HUMAN PANCREAS AND LIVER

MATHEMATICAL MODELING FOR NORMAL AND DISEASED STATE STUDIES

by

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ABSTRACT

Recent advances in biology, biochemistry, and medicine allow us to study – both qualitatively and quantitatively - the human body's response to a variety of perturbations. So, for example, we know quantitatively what will happen to insulin levels when a person is eats a meal; in many cases, we also know qualitatively the genes and signaling molecules will be stimulated or suppressed. These qualitative and quantitative data can be curated for statistical meta-studies and/or building mathematical models. Such mathematical models are made based on Transport Phenomena, Thermodynamics, Kinetics and PKPD (Pharmacokinetics and Pharmacodynamics) principles. This research is concerned with building mathematical models at the organ level (e.g. liver and pancreas), combining such organ models into a whole-body model such that we can better understand metabolism (or both normal and diseased people) under different conditions such as homeostasis, postprandium, exercise and so on. In particular, we have made a detailed, yet not too complicated, whole-body model to the body's response to glucose in normal people and in those with Type II Diabetes (T2D). Our results are presented in two parts. The other part is written by Hyun Park. In my M.S. Thesis, pancreatic α/β cell and liver organ models are explained using the mathematical tools such as Ordinary Differential Equations (ODEs), Flux Balance Analysis (FBA), optimization and sensitivity analysis. These models will show concentration and flux change of metabolites over time, parameter sensitivity and so on. Ultimately, the goal is to understand and model the body as a complex system with respect of components and interactions. With this we hope to understand the essential qualitative and quantitative features of T2D with the hope of developing new strategies for treatment of this disease.

Primary Reader: Marc D. Donohue

Secondary Reader: Michael J. Betenbaugh

PREFACE

Studying Chemical and Biomolecular Engineering at Johns Hopkins for the last two years for MSE degree has been full of challenges, surprises, fun and achievements. I have gained a lot of knowledge, met a great advisor and made many friends.

I would especially like to thank two people whom I met at Johns Hopkins and who will forever change my life. Professor Donohue and Hyun Park.

I would like to give me sincerest appreciation to my advisor, Professor Marc D. Donohue. Being an advisor to my research on Type 2 Diabetes and teacher Pharmacokinetics and Pharmacodynamics courses, he has done a tremendous job in enlightening me on Systems Biology. I learned about logical and problem-solving skills, biological and pharmacological systems and computational skills. With everything I learned from him, I will do my best to thrive in my career as a researcher.

I thank Hyun Park, my co-worker, friend and love of my life who has contributed so much to our research. With his kindness, encouraging natures, great work ethics and intelligence, my last two years have been full of bliss and fun. I am very glad he and I can continue career together at our next school.

Last but not least, both my beloved mother and father living in all the way across the ocean, in China, have supported me in thousand ways. I am always grateful and indebted to my parents.

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1. THE INTRODUCTION

This Masters Essay presents a comprehensive physiologic model of glucose uptake, metabolism, and storage in the human body. The purpose in building this model are:

(1) To organize the breadth of information on this topic into a coherent picture of glucose utilization; and

(2) To understand and model the complex behavior of glucose in the body with respect to all the key components and interactions involved in glucose utilization.

The goals of this research are to understand the essential qualitative features of glucose uptake, metabolism and storage, to correct errors in the conventional views of glucose behavior, as well as to develop new strategies for treating Type 2 Diabetes. The model developed covers the essential mechanisms that occur in the liver, brain, muscle, and adipose tissue. In addition to correcting errors in those models, it is hoped that the model presented here will expand on previous work by including physiological factors which, to best of knowledge, have not appeared in prior models of this system. In particular, in the model presented here differs in three ways from all prior models. They are: (1) Energy intake and expenditure are decoupled; fat intake leads to the production of ATP, but increased fat intake does not necessarily produce more ATP based on mass balance; instead, the amount of ATP a person consumes stays the same unless the person exercises. (2) Intracellular dissolvable and non-dissolvable metabolites (triglyceride and glycogen) are differentiated. Their dynamic behaviors are different; hence, it is necessary to devise separate equations instead of using the same equation. (3) Key enzyme interactions are determined using sensitivity analysis. Although a human body can convert one chemical into another as a chemical

reactor does, the actual process is much more complicated. One cannot build an in vivo model if only in vitro data are considered for each step due to enzyme interactions.

2. PREVIOUS EFFORTS IN BUILDING MODELS OF GLUCOSE UTILIZATION

2.1 Systems biology model

Systems biology is a biology-based interdisciplinary field that focuses on complex interactions within biological systems (Palsson, Bernhard 2015). It is a holistic approach to deciphering the complexity of biological systems. Systems biology uses mathematical models to help understand the dynamic process and reaction networks of living systems (Noguchi, R 2013).

E. coli is an organism that has been thoroughly studied. The reason most comprehensive modeling simulations have been done on E coli is that it is the best-characterized organism in the world. Feist (2008) and Palsson (2015) both did thorough information gathering on E coli systems biology modeling.

There is a process when it comes to building models for systems biology models like those for E. coli. It is based primarily on flux balance analysis, which includes: a) curating metabolic reactions, b) formulating the stoichiometric matrix (S matrix), c) applying mass balance constraints, d) defining the objective function(s), and e) optimizing the objective function using linear programming.

2.2 Human models

Over the past five decades, there has been considerable effort modeling glucose, fat and their regulatory hormones and these models have been used widely to help understand diabetes and related complications.

Combined circulatory and organ models of glucose and insulin interaction by Tiran (1980) have been developed based on a prior model of insulin and glucose by Tiran (1975). However, these

models do not show the detailed kinetics of how metabolites react and convert within an organ. In other words, they are not systems biology models. Sorensen (1985) developed an organ model for glucose metabolism by using features from different models, serving as a foundation for other models. Ajmera (2013) did a study of mathematical models of diabetes listing hundreds of models.

2.3 Human metabolite system biology model

The accomplishment of applying systems biology to bacteria like E. coli made it possible for researchers to develop detailed kinetics systems biology models for specific organs, and the liver has received the most attention. The liver, as the primary metabolic factory of the human body, is very important for storing and converting energy and for keeping the human body healthy.

Different hepatic models serve different functions and predict different trends in the role of hepatic glycogen regulation. Xu (2011) suggested a physiological model about glycogen metabolism in maintaining blood glucose homeostasis. Recently, Koenig (2012) presented a novel kinetic model of human hepatic glucose metabolism. This model includes glucose metabolic pathways in human liver along with the hormonal control of these pathways.

2.4 Discussion and what can be improved

There has been a significant increase in human systems biology modeling, particularly with respect to the increasing complexity of these models. However, there is still a huge gap between existing knowledge of physiology and the features of these mathematical models and there are deficiencies in all these models that can be improved. In particular, none of the existing models decouple energy (food) intake and energy consumption, many have errors in their physiology and enzyme kinetics, many lack optimization techniques and some even ignore conditions of homeostasis.

3. THE SCOPE OF THIS RESEARCH

The model presented here describes the relationships between glucose, fatty acid, amino acids, ketone bodies, intracellular metabolites such as G6P, F6P, glycogen, lactate, acetyl CoA and hormones such as insulin, glucagon and so on. To be more specific, the present model attempts to describe what happens to a normal human when the subject is fed, fasts, starves, is administered nutrients and exercises. The model also attempts to simulate how a patient with Type 2 Diabetes differs from someone without diabetes with the hope of elucidating the potential causes of T2D. To do this the model presented here must have a highly detailed whole-body model consisting of detailed organ/tissue/cell compartment models of a normal human with detailed systemic circulation, intracellular metabolism and signaling molecules.. Depending on what the focus is, some of the details can be lumped or disregarded. In most cases, signal molecules such as AS160, Akt, PI3 etc are omitted and genes such as HIF may not be considered.

A human whole-body compartment model consists of brain, heart, adipose tissue, muscle, liver and peripheral (rest of the organs/tissues/cells) organs/tissues/cells, each having its own metabolism and all of which are connected by arteries and veins. Also, under different conditions (mainly postprandial, fasting, starvation, administration of nutrients and exercises), simulating what happens to concentrations and reaction fluxes of macronutrients and metabolites inside the system and organs/tissues/cells is tried.

The primary focus of model presented here effort is to make a model of human physiology that is more realistic than previous models by decoupling energy intake and expenditure. Why this is important and distinct from previous models is because certain energy storage metabolites (energy intake) and energy expenditure (Basal Metabolic Rate-BMR- or exercise) are decoupled. Think of a car filled with gasoline (energy input) and engine's power (energy expenditure). In many

previous publications, the energy expenditure is dependent on the concentration of energy sources in the body; this would be the equivalent of a car's velocity being dependent on how full the gas tank is rather than on the pressure on the gas pedal. . However, physiologically (ie because of the decoupling of energy intake and energy expenditure) this is no more true in the human body than it is in an automobile. There are many mechanisms such as allosteric inhibition of enzymes or protein binding that stimulate or inhibit the metabolism of a species. While it may be true that storage of glycogen or TAG would accumulate as one consumes (energy intake) nutrients, it is wrong to say that TAG is used as an energy source because there is much TAG storage. In other words, just because one is obese, the obese person would not consume TAG at a higher rate.

To deal with this problem, in the model presented her, energy expenditure is decoupled from energy consumption. That is, to set TAG and glycogen energy expenditure to be independent of their concentration. Mathematically speaking, the energy expenditure of both species will be determined by basal metabolic rate and additional energy expenditure (i.e. exercise). The thermogenesis, digestive and miscellaneous energy expenditure are not considered here. Prior to this energy decoupling, stiffness was a major issue. That is, when ATP concentration, glycogen and TAG concentration were at extreme values, the model predicted that some of the metabolite concentrations became negative, reaction fluxes returned physiologically implausible values and optimized Km (Michaelis-Menten constant) and Vmax (maximum reaction rate constant) often returned unrealistic values.

When it comes to the physiology of energy decoupling, glycogen and TAG undergo reactions. Rather than being driven by glucose concentration, glycogen accumulates because of two major factors: first, the number of proteins called 'glycogenin' and second, the kinetics of glycogen accumulation (by insulin signaling, glucose and G6P) and degradation (by insulin signaling,

glucose and G6P as well). Previously, there has been considerable research describing glycogen metabolism with the latter reason only (kinetics). This may have been due to improper understanding of glycogen metabolism or knowing simplification of the equations. This model presented here simplifies glycogen metabolism but with a better understanding of this issue. The detailed explanation for glycogen metabolism is presented below.

Glycogen molecules have central proteins called glycogenin which exist in certain amount (may be due to thermodynamic equilibrium) and this is the reason why there are multiple glycogens detected in liver and muscle cells.

As for TAG, there are proteins called 'perilipins' which are on the surface of lipid droplets - primarily in adipose tissue cytoplasm. Lipid droplets are clusters of TAG. Once TAGs are absorbed into the white adipose tissues (WAT), they cluster together forming a large lipid droplet organelle which pushes the rest of the organelles such as endoplasmic reticulum, nucleus, ribosomes and so on to the side. Hence, WAT cells grow in size (hypertrophy) rather than growing in number (hyperplasia) as lipid droplets collect more TAGs and vice versa. The lipid droplets, however, are not under pressure to go through TAG releasing metabolism just because of its concentration. The perilipins hinder lipid droplets from degrading at high concentration. Perilipins undergo conformational change to release TAG from lipid droplets, only upon exercise, nutritional conditions (fasting etc.) or hormonal actions.

4. PHYSIOLOGICAL RESEARCH FOR WHOLE-BODY MODEL

Knowledge of human physiology is not yet developed to the point that one could create a model to treat all human physiology and its variation from organ to organ over the whole range of physiologic conditions. Therefore, innumerable assumptions and simplifications necessarily must be made. Depending on how detailed or broad one would like to make a whole-body, multi-compartment model, there are innumerable different possibilities of what the end model would look like. For example, there have been some previous attempts at making an endoplasmic reticulum (ER) subcompartment and a mitochondrial subcompartment within intracellular cytoplasm compartment (or organ/tissue compartment). The reason for having a mitrochondrial subcompartment is that there are several metabolites whose concentrations in cytoplasm and mitochondrial matrix are quite different. For the ER, there is a concentration difference of calcium ions between the ER and cytoplasm due to calcium sequestration and release by the ER.

On the other hand, apart from detailing the 'compartments', one also may consider the 'level' of variables. Components which are involved in metabolic network categorized. There are four categories one could think of: macronutrients, intracellular metabolites, signal molecules and transcription-level agents.

Macronutrients are, in simple definition, chemicals which can be detected in the system (i.e. blood circulation) as a result of administration into the body for energy use or secretion from the organs/tissues/cells. Examples include derivatives of carbohydrates, fats and proteins such as glucose, fructose, fatty acids, triacylglycerides, amino acids, pyruvate, lactate, glycerol, etc.

Metabolites, in simple definition, are chemicals which can be only detected in the cytoplasm which normally do not exit the organs/tissues/cells. They mostly appear inside the organs/tissues/cells

due to the organs/tissues/cells' specific metabolism. For example, glycogen is present primarily in liver and muscle. Though it can also appear in adipose tissues as well, glycogen is not present in blood or plasma. Glucose-6-phosphate is another example of metabolite produced via glycolysis or gluconeogenesis but never leaves the cell. One should bear in mind that such intracellular metabolism is driven by (mostly protein) enzymes. The enzyme effect is indirectly expressed as Vmax and directly as Km.

Signaling molecules are non-metabolite nor non-enzyme molecules which are involved in transduction of signals to make cellular metabolism possible. Some examples are IRS, Akt, AS160 etc. IRS is an abbreviation for Insulin Receptor Substrate and is a protein which binds to the β (intracellular) portion of IR (Insulin Receptor). The β IR undergoes conformation change upon insulin binding to the α IR, then IRS binds to β IR. IRS binding to β IR will trigger and propagate numerous downstream reactions including PI3K reaction, phosphorylation, AS160 reaction etc. Akt is one of the downstream reaction protein involved in transduction and stimulation of other signal molecules. AS160 perhaps is responsible for and involved in GLUT4 translocation onto the surface of the cellular membrane (of muscle, usually). There will be more detailed explanations in later section in this chapter.

Lastly, transcription-level agents are chemicals involved in genetic level. They control long and short term changes of organs/tissues/cells and produce enzymes, signal molecules etc. DNA, mRNA and transcription factors are such examples.

In this model, for simplicity without losing much detailed complexity, macronutrients, metabolites and a few signaling molecules were included.

4.1 Blood macronutrient concentration

In this model, the following macronutrients are going to be considered. Glucose, Free Fatty Acids (palmitate is used to represent the wide variety of FFA), AA (alanine, gluconeogenic amino acid), KB (representing general group of ketone bodies, partially oxidized FFA and can only be used in cells containing mitochondria: i.e. erythrocyte cannot use KB such as β-hydroxybutyrate and acetoacetate), glycerol (stoichiometry with fatty acids is important), TAG, lactate and pyruvate. Detailed metabolism of these enumerated metabolites will be explained for different organs/tissues/cells in the later part of this chapter.

Nutrients	Days of Fasting (0)	1	2	3	4	5	6
Concentration							
Glucose (mM)	3.67	4.67	4.05	3.78	3.61	3.67	3.44
Free Fatty Acids (mM)	0.53	0.42	0.82	1.04	1.15	1.27	1.18
Ketone Bodies (mM)	0.00	0.03	0.55	2.15	2.89	3.64	3.98
Glycerol (uM)	0.00	62.00	85.00	95.00	91.00	100.00	76.00
Insulin (pM)	458.33	583.33	506.94	472.22	451.39	458.33	430.55

Table 4.1 1 Blood nutrients level in 6 days fasting

4.2 Organ/Tissue/Cellular metabolites

For metabolites, the research considered the followings. G6P (glucose-6-phosphate), glycogen, F6P (fructose-6-phosphate), G3P (glycerol-3-phosphate), OAA (oxaloacetate), AcCoA (acetyl coA), NADH, NAD+ (Nicotinamide Adenine Dinucleotide), FADH2, FAD+ (Flavin adenine dinucleotide), GTP (Guanine Triphosphate) and GDP.

G6P is the very first metabolite in this model under glycolysis. Under gluconeogenesis (in liver and kidney only), G6P will convert to glucose. Glycogen is a polymer metabolite which stores glucose monomers. F6P is a metabolite converted from G6P and both G6P and F6p are in thermodynamic equilibrium by a single enzyme responsible for the reversible reaction. G3P is a metabolite which can be produced from F6P via multiple reactions, which are lumped in this model. In this case, multiple biochemical reactions are lumped into one simplified hypothetical reaction represented by 'phenomenological' Vmax and Km, rather than experimental values. G3P can also be produced from glycerol and be used to produced TAG with fatty acids (stoichiometry, G3P:FFA=1:3). Pyruvate is a metabolite produced from G3P via multiple reactions and in this model, such reactions are lumped into one reaction. Pyruvate can also be produced from lactate and vice versa due to an enzyme responsible for such reversible reaction. Pyruvate is a versatile metabolite because it can produce many important metabolites which can be used for TCA cycle, which is necessary for generating ATP under aerobic condition through electron transfer and proton gradient. Two pyruvate-produced metabolites which are thought to be important in this model are OAA and AcCoA. OAA is known as a metabolite which is used as an intermediate reactant alongside AcCoA to activate TCA cycle while OAA also can generate G3P. AcCoA is responsible for generating multiple ATPs in mitochondria due to its biochemical reaction where it

can break down up to 8 times. If for any reasons either OAA or AcCoA depletes, TCA cycle will be affected. KB can also be generated from AcCoA and vice versa in some organs/tissues/cells.

One should keep in mind that some of the intracellular cytoplasmic metabolite also can exist in some of the organelles such as mitochondria, a subcompartment. In that case, the concentration of the coexisting metabolites may have different concentration in both places or it may be very hard to measure the subcompartmental concentration. In such cases, one can choose to disregard subcompartmental concentration or assume concentration based on educated guess. In this model, subcompartmental concentration is disregarded for simplicity and because it does not affect this model much because of the scale of this model. Likewise, due to less importance, pancreatic ER subcompartment where it introduces interesting Hodgkin Huxley-like calcium based electro-oscillation is omitted. It is important to mention that some of the organ specific models (Hodgkin Huxley being one of them), can be simplified so that oscillatory behaviors in this case, can be ruled out in the whole-body model. Order of magnitude analysis (OMA) says that oscillation is less important in oscillatory effect versus convection of blood effect.

When it comes to physiological (both clinical -in vivo- and experimental -in vitro-) data of macronutrients and certain metabolites, there are decades' worth of studies (mathematical equations, plots, data points etc) accumulated in multiple journals, dissertations and books. Here are some of the study types identified which have repeatedly been shown throughout the literature search.

Macronutrient concentration over time under postprandial, fasting and exercise 'conditions' (denoted below as 'conditions') of healthy or diseased patients. This study type elaborates on macronutrients concentration change once an individual is administered (usually orally) macronutrients. For example, in the simplest case, if one is injected pure glucose in the system,

that person will experience sharp glucose concentration at the beginning (near time of administration, but not exactly t=0 due to blood circulation) and glucose concentration will decrease over time due to the body's glucose utilization and clearance mechanisms.

Certainly, there are inter-individual variability of how much glucose can be distributed, utilized and cleared from the body due to gender, health, age etc. However, the goal of this model is to make an ideal healthy patient model and then generalize this model to better understand the development and evolution of T2D. Moreover, when a patient consumes a meal, the patient will experience that the concentrations of certain macronutrients change as time passes. However, one should keep in mind that in uncontrolled environments, no two meals a person eats are exactly the same. For instance, people do not eat exactly 40% glucose (carbohydrates), 30% palmitate (FFA) and 30% alanine (AA) of 200g of food in every meal, not to mention that there are numerous types of carbohydrates (fibers, sugar, lactose…), FFA (poly/mono/ (un)saturated FA), AA (leucine, histidine …) and additional nutrients consumed. In the publications studied, however, the patients were given controlled amounts of food so that the input was fixed (food administered) but the outputs (concentrations of macronutrients) necessarily were variable.

Using such controlled studies, the research also could simulate the input and output relationship as reported in the articles; moreover, the research also could simulate the food administration of different composition situations, not having been researched in any of the articles. Likewise, one can use different 'conditions' other than the postprandial state. There are studies conducted on individuals who exercise at different levels (expressed as percentage of VO2max) and duration (expressed as time) or those who fast for a certain amount of time. If this model can simulate every condition reported in the publications with certain confidence level, the prediction on what a human body model will go through will be even more accurate.

Blood flow change and flux (uptake and release rate) of macronutrients into and out from between blood vessel and organs/tissues/cells under different conditions. The fundamental principles in this study type are same as macronutrient concentration versus time study. For example, using a controlled meal plan like that mentioned in macronutrient concentration studies discussed above, one could also measure how much the blood flow of arterial/venous blood vessels and absorption/secretion rates of organs/tissues/cells change: e.g. to measure lactate secretion rate when fasting. Also, studies under different 'conditions' and blood flow and flux change are reported.

Hormonal level and flux (hormones such as insulin, glucagon, adrenaline etc.) change over time under different 'conditions'. The fundamental principles in this study type are same as macronutrient concentration versus time study. This is usually done with the first type of study (macronutrient level and flux).

Specific organs/tissues/cells' metabolism (concentration and flux of metabolites) under different 'conditions'. The fundamental principles in this study type are same as macronutrient concentration versus time study. One example is to feed patient a designed meal to measure glycogen concentration in liver or muscle.

Concentration and flux change of macronutrients, metabolites and hormones under 'controlled' condition. This study type is slight different in that the focus in this research is to find relationship between controlled concentration or flux of macronutrients, metabolites and hormones and those uncontrolled. For example, hyperinsulinemia is a well performed and recorded study where the researchers maintained the level of insulin from basal level to manifolds higher and sought how glucose secretion rate is affected. Another example is to infuse glucose at different rate to measure insulin secretion rate. This type of research may be reported as concentration vs rate instead of

time as observed in the previous study types. This type of controlled study would give this model more robustness for parameter tuning.

Finally, different models have different purposes. There is a saying that all models are wrong but some are useful. The most basic (does not mean it is easy) type of model is to simulate concentration and flux over time without blood flow or hormonal levels being affected under different 'conditions'. One should keep in mind that when one eats or exercises or in different health condition, not only would the basal concentration and flux of macronutrients and metabolites would be affected but also the hormonal level, blood flow or oxygen level (which is disregarded in this model) would also be affected. In other words, to make a more precise model, one should consider all of the factors mentioned. For example, when a person exercises, it will affect insulin/adrenaline/glucagon level, which will affect blood flow rate and concentration and absorption/secretion rate of macronutrients and metabolites. The effect of glucose on concentration/flux of glucagon can be expressed as constitutive equation for simplification or as part of ODEs for precise modeling.

Reaction enzyme	Positive regulation	Negative regulation
Glucokinase		F6p
Phosphofructokinase	AMP	Citrate
Glycogen phosphorylase	AMP	Glucose
Ga3p dehydrogenase		Glucose
Pyruvate kinase	F16p	Amino acids

Pyruvate dehydrogenase		NADH, ACoA (Pi)
Citrate synthase	AMP	
Isocitrate dehydrogenase		SCoA (Pi)
AKG dehydrogenase	AMP	SCoA (Pi)
Citrate shuttle103		PalCoA (Pi)
Cit_ACoA_OAA (ATP citrate lyase)		PalCoA (Pi)
ACoA_Mal-CoA (acetyl CoA carboxylase)		PalCoA (Pi)
FFA_PalCoA (acyl CoA synthase) (Saggerson, 2008)		Mal-CoA (Pi)
PalCoA_ACoA (b oxidation)		ACoA (Pi)
Carnitine shuttle (carnitine acyltransferase)		Mal-CoA
Gmt_AKG (glutamate dehydrogenase)		FFA
ACoA_Gmt_NAG (N-acetyl glutamate synthase)	Arginine	
NH4_Crbphos (carbomyl phosphate synthase)	NAG	
Citrulin_Arg (argininosuccinate lyse)	AMP	
(Glucosamine 6 phosphate N-acetyl transferase)	FFA	Glnac (Pi)
(N-Acetyl glucosamine pyrophosphorylase)	Glucose	
HMG-CoA_Mevl (HMG-CoA reductase)		Mevl (Pi)

Table 4.2 1 Key enzymes and their regulators

4.3 Signaling molecules

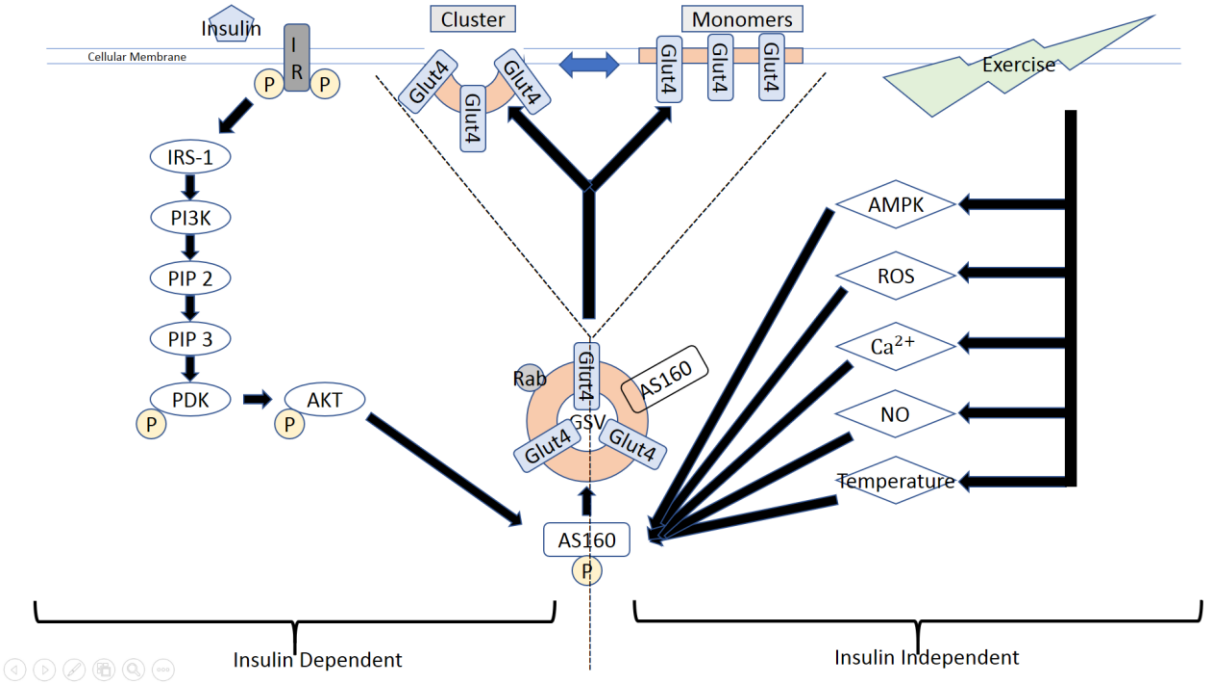

Figure 4.3 1 GLUT4 translocation via insulin in/dependent pathways

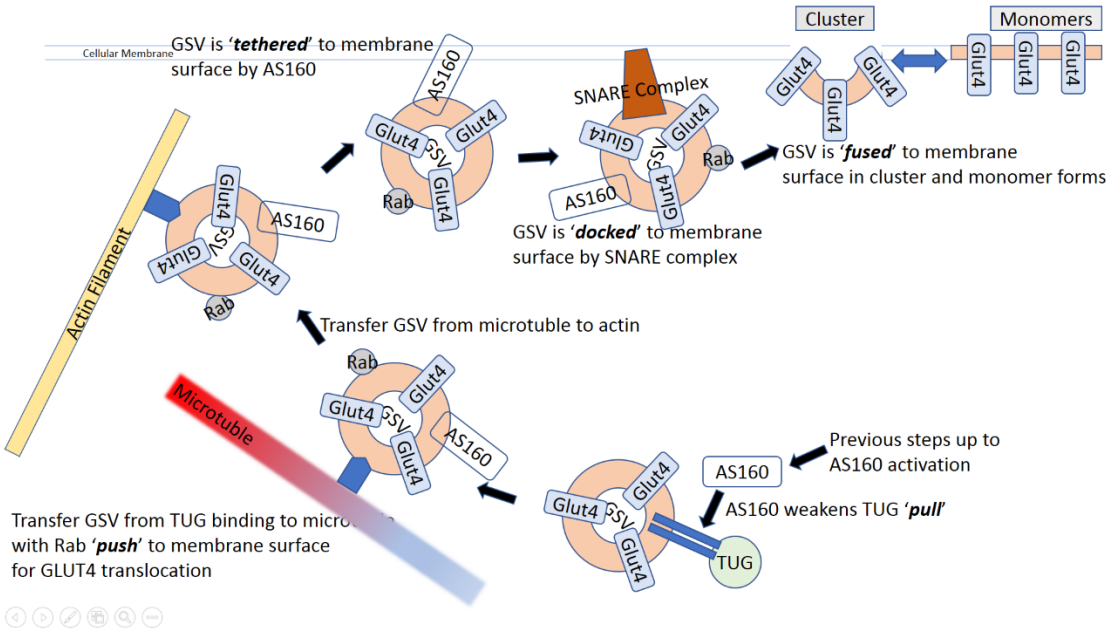

Figure 4.3 2 GSV delivery mechanism

There are multiple signal molecules involved in the cascade (transduction) of signals under different conditions. The one of the most widely studied cases involves insulin (controlled condition) vs physical exercise (different 'conditions') signaling effect of GLUT4 translocation (this is not part of concentration/flux study type enumerated above because GLUT4 is not a metabolite but the end product of signal molecules transduction). GLUT4 can, therefore, be thought as part of signal molecule 'level'.

GLUT4 translocation is thought to be important because GLUT4 is one of the main transporters in a human body and is thought to be responsible for controlling blood sugar level. Transporter and channels are proteins which let macronutrients and other chemicals to enter or exit the cells so that metabolism can happen. When metabolism happens inside the cells, there are enzymes and signal molecules (with transcription 'level' activities) which are playing roles. GLUT4, lets glucose get in and out from the cell. MTC lets lactic acid to get in and out. However, the reason why there has been more study of GLUT4 is because glucose absorption and secretion are directly related to GLUT (1 and 4) transporters. Therefore, there are more studies regarding GLUT4 translocation than any other transporters in T2D studies. Whether GLUT transporters and especially GLUT4 are truly responsible for T2D are still open to debate. In this model, even though GLUT4's activity matters in leading to T2D, the primary cause of T2D onset is thought to come from visceral adipose tissues. This will be explicated in chapter 12.

Here are a few more pieces of information on GLUT4. GLUT4 resides in 50-70nm GSV (majority, scattered throughout cytoplasm) and larger structures, being derived from trans-Golgi network and endosomes. In an effort to identify GSV specifically, insulin-regulated amino peptidase (IRAP) was devised. GLUT4 and IRAP are highly colocalized while v-SNARE VAMP2 not in GSV (however, IRAP and VAMP2 are both abundant in GSV). GSV varies in GLUT4 content

(heterogeneous). IRAP-pHluorin and VAMP2-pHluorin can be used (prior on GSV GLUT4 and latter on GSV (56nm) and larger structures (150nm)). Within 3-4 minutes after insulin stimulation (insulin burst) IRAP-pHluorin shows rapid fusion of GSV while VAMP2 shows two events (burst-GSV- and continuous-larger structures-).

Rate of entry of glucose into cells is regulated by transporters. Blood glucose level is 5mM. Basal Glucose transporter in most cells has Km of 1mM. Less than blood glucose and so glucose is taken up easily. But in liver and pancreas glucose transporters have Km of ~15mM (close to blood glucose levels). This allows pancreatic cells to monitor glucose levels and thereby regulate insulin secretion. In liver cells, glucose is only taken up when it is very abundant. Then liver cells acquire glucose and convert it to glycogen and fatty acids.

Putting the controversy concerning GLUT4 aside, two main ways in triggering GLUT4 translocation will be discussed: insulin and exercise.

Insulin is more obvious reason for GLUT4 translocation. Insulin is secreted from pancreas when glucose (mostly) or AA (low to moderately) sources are detected by the pancreas. Then the pancreas releases insulin, which will stimulate certain organs/tissues/cells to absorb glucose. This research is going to use an example of muscle and is not going to differentiate various muscles in favor of simplicity.

Muscle absorbs glucose using GLUT4 when insulin binds to its sarcolemmal membrane's Insulin Receptor (IR) α component. This will change conformation of β component of IR, which is inside the cell. This will attract Insulin Receptor Substrate (IRS) which will activate PI3 Kinase (PI3K). PI3K will then affect PIP2 and PIP3. PIP3 will affect PDK, which will activate Akt. Akt will in the end activate AS160 (a.k.a. TBC1D4). AS160 will then affect GLUT4 vesicles to be merged with inner cellular membrane so that GLUT4 can be translocated on the cellular

membrane. This process is still not entirely known and this research will cite one publication to elaborate what are thought to happen during this process. AS160 protein and rest of the process taking place is important and interesting because exercise induced GLUT4 translocation happens to have the same process (before which the transduction process is different) once it reaches AS160.

In many of the articles, AS160 affecting GLUT4 delivery to membrane surface process is still unexplained. It is hypothesized based on [ref] and a publication regarding SNARE protein, that AS160 brings the GLUT4 vesicle to the SNARE complex, which is responsible for snatching the vesicle and fusing the vesicle with the cellular membrane (Sonntag, Annika G 2012).

SNARE complex is a protein structure consisting of multiple smaller proteins: VAMP2, Syntaxin-4 and Munc18-c. When these structures are assembled to form a SNARE complex, the complex is responsible for any type of exocytosis behaviors of cells. The reason why it is exocytosis-ish is because in GLUT4 translocation, GLUT4 vesicle is snatched by SNARE and the vesicle is fused in the membrane so that GLUT4 are placed in the middle of bilayer membrane proteins. That is, GLUT4 are not secreted. In case of pancreatic β cells, where SNARE complex snatch insulin containing vesicles and fuse them with the β cell membrane, the insulin is secreted. Therefore, this is exocytosis-like behavior.

The articles suggest that GLUT4 vesicle is transported by microtubule then it is transported to actin. GLUT4 vesicle has AS160 and VAMP2 imbedded in the vesicle layer alongside Rab and GLUT4 proteins, when it is moved to the inner cellular membrane during the transportation. Whether AS160 and VAMP2 allure the vesicle to formation of SNARE complex is unknown; however, it is assumed so. After the GLUT4 vesicle approaches the membrane surface, SNARE complex is assembled which fuses the vesicle to the cellular membrane.

Isoform	Tissue Distribution	Afinity for Glucose	Km (mmol/L)	Features
GLUT1	Brain microvessels Erythrocyte Placenta Kidney All tissues	High	1	Ubiquitous Basal transporter
GLUT2	Liver Kidney β cell Small intestine	Low	15-20	Insulin independent
GLUT3	Brain neurons placenta Foetal muscle All tissues	High	<1	Found in glucose dependent tissues
GLUT4	Muscle cells Fat cells Heart	Medium	2.5-5	Insulin dependent
GLUT5	Small intestine Testes	Medium	6	High affinity for fructose

Table 4.3 1 Glucose transporters

The signal molecules listed here have been known to play central role in insulin triggered GLUT4 translocation. The individual signal molecule's exact role is still being studied and modified with additional proteins being discovered, disregarded and discarded in the transduction process meanwhile. There are many studies which say the disruption of certain metabolites, enzymes or signal molecules are the main reasons for onset of T2D (experimented by artificially encoding genes to be knock-out of certain chemical of interest or measuring the level of certain chemicals of interest etc.); however, the onset of T2D is still undetermined. Disruption/alteration in signal molecule's activity/concentration (IR to SNARE complex) may not be because they are the cause of T2D but because they may be merely biomarkers of T2D (byproducts as part of the T2D process). In other words, there may or may not be correlation but necessarily causation between onset of T2D and signal molecule's activity/concentration deviation (Sonntag, Annika G 2012).

Another main way for triggering GLUT4 translocation is exercise. The similarity between insulin induced translocation and exercise induced translocation, as mentioned briefly above, is that both mechanisms share downstream process after AS160 protein is activated. Before the activation of AS160, the signal molecules and reactions taking place are different from insulin induced translocation. There are a few factors which are thought to cause pre-AS160 process. AMP/ATP ratio, ROS (Reductive Oxidative Species), calcium ion, NO (Nitrogen Oxide), temperature etc. Unlike insulin induced GLUT4 translocation where multiple signal molecules being involved in a single long chain of reactions, exercise induced GLUT4 translocation has multiple (parallel) starting points, hence many short chains of reactions until they reach common convergence, i.e. AS160. Everything except AMP/ATP ratio will be explained further in muscle part of this chapter.

AMP/ATP ratio plays the following role. When one exercises, there will be fluctuations of AMP, ADP and ATP concentration. Assuming exercising without consuming any food, at the beginning, the residual ATP will be used, then creatine/phosphocreatine (CR/PCR) will generate ATP for a short period of time. Afterwards, glycogen storage will release glucose which will be used for glycolysis for anaerobic exercise. The first three (residual, CR/PCR and glycolysis) are anaerobic by nature and do not need oxygen for production of ATP. As a result of anaerobic exercise, pyruvate turns into lactate (oxidation taking place in TCA cycle is not quickly able to utilize pyruvate so some pyruvate changes into lactate) and the lactate cannot be used in muscle so it has to be released and absorbed by liver. Once absorbed by liver, depending on the necessity, the lactate will convert back to pyruvate and can be stored into glycogen or undergo gluconeogenesis (especially when exercising) or be used for TCA. This will be explained in chapter 6. Under aerobic exercise and resting condition, TCA cycle will have enough time to produce extra 34 ATPs utilizing FFA or KB, which will convert to AcCoA and be incorporated into TCA. The definition of aerobic and anaerobic will be explained in chapter 6. Either way, ATP will be generated. When exercising, ATP will be used for BMR, thermogenesis and exercise. Such extra energy expenditure in the form of exercise is to fuel muscle's movement such as stretch and contraction. Breaking homeostasis of AMP/ATP ratio, AMP will increase due to higher energy expenditure when exercising. This higher ratio will in turn activate AMPK (AMP Kinase), which is a protein responsible for numerous reactions, not just limited to muscle's exercise induced GLUT4 translocation. AMPK will activate AS160 and probably the same process mentioned in insulin induced GLUT4 translocation will happen.

So far, the research has introduced what happen to GLUT4 translocation arising from insulin and exercise effects, to explain signal molecule 'level'. There may be different signal molecule

reactions happening for glucagon/adrenaline binding on muscle/other organ/tissue/cell receptors and under resting/exercise/fasting/postprandial conditions. With limited resources and time, it would be challenging to incorporate all of such information into one thesis. However, such information is of absolute necessity to fully understand the signal molecule process.

Signal Molecules	Positive regulation	Negative regulation
IRS		PTP, PKC, S6K
AKT	mTORC2	Glnac, TRB3
PKC	DAG, Glnac, FFA	
GSK3	PP1, Phk	Cal, PKA, FFA
mTOR	Amino acids	
S6K	Amino acids	AMPK
TSC	AMPK	AKT
cAMP	Gprt	PDE3
PKA	cAMP	
PDE3	AKT	PKA

Table 4.3 2 Signaling molecules and their regulators in GLUT4 transport pathway

4.4 Transcriptional level

In most of the models, transcription level or gene level are not usually described. It is usually because simpler models are easier to make and also transcription level are for the long term effect which could take up to a few hours. For the modelling purpose, transcription level is not necessary. In this model, however, one transcription factor and its gene out of many other transcription level chemicals, called Hypoxia Induced Factor (HIF-1) are introduced. Other transcription level chemicals and their corresponding genes often found in experimental data in T2D and related diseases (such as atherosclerosis, high cholesterol etc.) are FOXO (Forkhead Box Protein), SREBP (Sterol Regulatory Element Binding Protein) and CHERP (Calcium Homoeostasis Endoplasmic Reticulum Protein) etc. The main reason why such transcription level chemicals and genes are related to not only T2D but also other diseases is because many of transcription level chemicals and genes share same metabolic pathways for normal human beings. That is, for diseased humans, some parts of the transcription level processes in the same metabolic pathway malfunction while other processes may or may not function normally. Therefore, SREBP, which is responsible for high cholesterol disease due to its cholesterol controlling mechanism, play a role in leading to T2D because FFA concentration in T2D patients also change. However, the research would like to introduce a transcription factor (HIF-1) which is more unique and thought to be fundamental to T2D.

Two snippets of information regarding HIF-1 and T2D research are introduced here.

For the first part of the information, it has been long thought that T2D patients are obese and the obesity is the driving force for insulin resistance. To further explain how obesity induces insulin resistance, research on HIF-1 alteration in adipose tissues and the adipose tissues' changed

metabolism somehow causing insulin resistance were introduced. However, a few weaknesses in this explanation pointed out.

First, the definition of obesity is not clear and there are different types of obesity. In many of the old to recent articles, the experiments performed on patients to measure their obesity has been surprisingly wrong. An individual is defined to be underweight/lean/overweight/obese depending on Body Mass Index (BMI). BMI is a function of height and weight. It is easy to think that for the given gender and age and height, if one's weight is appreciably high, that person is thought to be obese. The usual image of obese people is those who have portending belly and with extra fat on limbs. This, however, is an image limited to subcutaneous fat. Subcutaneous fat are adipose tissues sitting under the skin and is the main reason why people become obese. This type of fat has less constriction in terms of how and where it can expand/shrink. However, there are people who look lean but in fact are more likely to have T2D and cholesterol related diseases. This group of people tend to have more visceral (around organ) fat. This type of fat is relatively more confined in terms of movement. Those who possess high visceral fat may have lower BMI. Therefore, it is necessary to measure subcutaneous/visceral fat when it comes to T2D studies.

Second, the conventionally defined obese people do not necessarily have T2D. There are group of people who have high BMI but are metabolically healthy. This group of people is called MHO (Metabolically Healthy Obese). They, indeed, will be less likely to have T2D or cardiovascular related diseases.

Third, the mechanism in HIF-1 expression altered adipose tissues' metabolism causing 'insulin resistance (probably muscle)', 'energy expenditure' and 'β cell glucose intolerance' needs to be understood better (Gunton, Jenny E 2005). One should bear in mind that correlation is not causation. HIF-1 may or may not be the direct cause of the listed phenomena, so it is premature to

say that HIF-1 is cause. For example, if HIF-1 is the cause of insulin resistance, how the metabolism in insulin resistant organs/tissues/cells changes as a result of HIF-1 expression alteration in adipose tissues should be thoroughly explained.

For the second part of the information, there were a few different researches in vivo/in vitro experiments on humans and animals to measure HIF-1 level and the corresponding metabolism change in different organs/tissues/cells (Girgis, Christian M 2012). However, this also comes with a few weaknesses.

First, in vitro data may not be applicable to in vivo situations. This is well known but oft-disregarded fact. Up to these days, many researchers use in vitro data to fit their models' parameters. There should be justification and study on how and when in vitro can be used for in vivo.

Second, how the HIF-1 level changes in different organs/tissues/cells under healthy or T2D condition is not well understood. HIF-1 level change in different cells extracted from different organs/tissues/cells are studied (Wang, Xiaohui L 2009). It is one thing to study individual cell's HIF-1 expression change and its metabolism changes and it is another to study how different cells from different organs/tissues/cells affect each other which lead to HIF-1 change and T2D.

Third, in case of muscle and adipose tissues, the exact definition of them is required. Muscle can be categorized in different ways. White muscle and red muscle, active muscle and inactive muscle when exercising etc. In many researches, one of the thigh muscle is well studied due to its high involvement in motions. If one generalizes muscle, the model result may not be accurate. Same goes for the adipose tissues. Subcutaneous, visceral and ectopic fat, white adipose tissue (WAT)

or brown adipose tissue (BAT) are very different. Normally, WAT is what we refer to as fat in subcutaneous and visceral fats. BAT is near spine, shoulders, neck and back.

Fourth, adipose tissue acting as a master regulator of bodily metabolism is suggested in many articles. An explanation for this may give an answer T2D propagation, which is not available in any previous research. This is an extension of the second point of second snippet. Many articles point out that fat is the master regulator for body's metabolism change in T2D and other conditions. It is necessary to prove how the adipose tissue plays this role.

There are several ideas revolving around the onset of T2D. This will be explained in later chapter 12. The thoughts on how HIF-1 is related to T2D is explained in chapter 12. However, this chapter will explain what HIF is first.

Hypoxia inducible factors (HIFs) are master-regulators of cellular responses to hypoxia, and thus are crucial for survival. HIFs also play a role in regulating cellular processes in β-cells, liver, muscle, and adipose tissue, have effects on the regulation of weight, and play a role in type 2 diabetes (T2D). HIF target genes, including genes encoding proteins involved in angiogenesis, apoptosis, cell cycle progression, glucose uptake, glycolysis, and lipid metabolism. Composed of two parts (α and β – β is called ARNT). Two major risk factors for T2D are insulin resistance and β cell dysfunction. Disturbances in HIF-1 signaling may play a detrimental role at several stages in diabetic pathogenesis, including the innate failure of β cells to secrete sufficient insulin, insulin resistance, adipocyte dysfunction etc (Pillai, Renjitha 2015).

In pancreas, islets isolated from people with T2D have a 90% decrease in ARNT expression. β cell ARNT KO (β ARNT) had reduced glucose tolerance and impaired in vivo and in vitro GSIS, and similar changes in gene expression to those seen in T2D islets, including decreased HNF4a, insulin

receptor, aldolase, phosphofructokinase and others. β cell culture models (MIN6) shows consistent result. Significantly impaired insulin secretion, reduced glycolytic enzyme expression, and dysregulation of metabolic pathways. Carbohydrate-responsive element-binding protein (ChREBP) as a negative regulator of ARNT and has suggested that ChREBP-mediated repression of the HIF complex might contribute to glucotoxicity induced β cell dysfunction. β-HIF-1a null mice had severely reduced glucose-stimulated ATP generation and therefore impaired insulin release. VHL is required for HIF proteolysis, and thus deletion or inactivating mutations increase HIF protein. Interestingly, mice with β cell deletion of VHL have markedly impaired insulin secretion and glucose; both depletion of ARNT or HIF-1a and excess of HIF-1a and HIF-2a (with are both increased with VHL deletion) impair β cell function, suggesting an 'inverse U' relation. Use of iron chelators resulted in improved insulin secretion and normalized ARNT mRNA and downstream gene expression in islets from people with T2D. Deletion of FIH, which causes relatively modest increases in HIFs, also results in improved glucose tolerance in mice challenged with high-fat diet (HFD). Human islets from people with T2D have decreased expression of both HIF-1a and ARNT, and increasing HIF-1a with iron chelation improved human islet function and gene expression (Yamaguchi, Miwa 2015).

In liver, liver dysfunction is a key component of T2D and is both affected by and contributes to the condition. Cardinal features include increased and inappropriate hepatic glucose production (HGP) and reduced hepatic insulin sensitivity. Non-alcoholic fatty liver diseases (NAFLD) are common in T2D and further exacerbate metabolic dysfunction. It has been shown that feeding mice with a high-fat/sucrose diet that causes fatty liver also causes upregulation of hepatic HIF-1a. When mice with a hepatocyte specific HIF-1a deletion were fed this diet, they exhibited more severe impairment of glucose tolerance and peripheral insulin resistance. Consistent with the

decrease in ARNT mRNA in T2D islets, ARNT mRNA and protein are also reduced in human T2D livers. Short-term hepatic ablation of ARNT in mice using adenovirus injection increased HGP and impaired glucose tolerance. Conversely, deletion of hepatocyte VHL substantially increased hepatic HIF-1a and HIF-2a proteins and caused life threatening hypoglycemia and reduced ketones (Yamaguchi, Miwa 2015).

In muscle, HIF-1 plays a role in this dynamic process by regulating glycolytic and oxidative pathways of energy production, mitochondrial respiration and muscle fiber composition. Its role is suggested by three observations: expression is higher in fast twitch muscles that rely on glycolysis and is upregulated during bursts of activity and in chronic hypoxia. HIF-1a upregulated GLUT4 mRNA after 10 min of electrically induced contraction of isolated soleus muscle; thus HIF-1a might facilitate glucose transport following contraction (Yamaguchi, Miwa 2015).

In adipose tissues, obesity is the strongest acquired risk factor for T2D: it gives a 10-fold higher risk in men and 30-fold higher risk in women. Adipose tissue hypoxia, and this appears to drive an inflammatory response. In obese humans, reduced oxygen pressures within abdominal fat associate with greater macrophage infiltration. Collectively, these observations suggest that (i) adipose tissue hypoxia is deleterious and (ii) that defects in the adipocyte response to hypoxia may contribute to the pathogenesis of insulin resistance and diabetes. Adipocyte-specific HIF-1a and adipocyte-specific ARNT knockout mice report the protection of these mice from the consequences of HFD. Specifically, these mice were resistant to weight gain, and had substantially better glucose tolerance and insulin sensitivity. Increased insulin-stimulated Akt phosphorylation was seen in WAT, liver and muscle in adipocyte specific HIF-1a knockout mice, suggesting crosstalk between adipose tissue and other sites. These mice also displayed central effects with an increase in core temperature and energy expenditure (Yamaguchi, Miwa 2015).

Transcription factors	Positive regulation	Negative regulation
SREBP	S6K, AKT, PKC	cAMP, FOXO, AMPK
ChREBP	Glucose	PKA, AMPK
PPARg	AKT, FFA	AMPK
PPARa	PKA, FFA, PGC	
CREB	PKA	AKT
CEBPa	cAMP	PKC
TRB3	PI3K, PKC, PPAR, PGC1	
PGC1	FOXO, CREB	AKT
FOXO	Glnac, AMPK	AKT, PPARg
AMPK	AMP	AKT, PKA, ATP

Table 4.3 3 Transcription factors and their regulators

5. BLOOD/ECM/ORGAN CELL COMPARTMENT

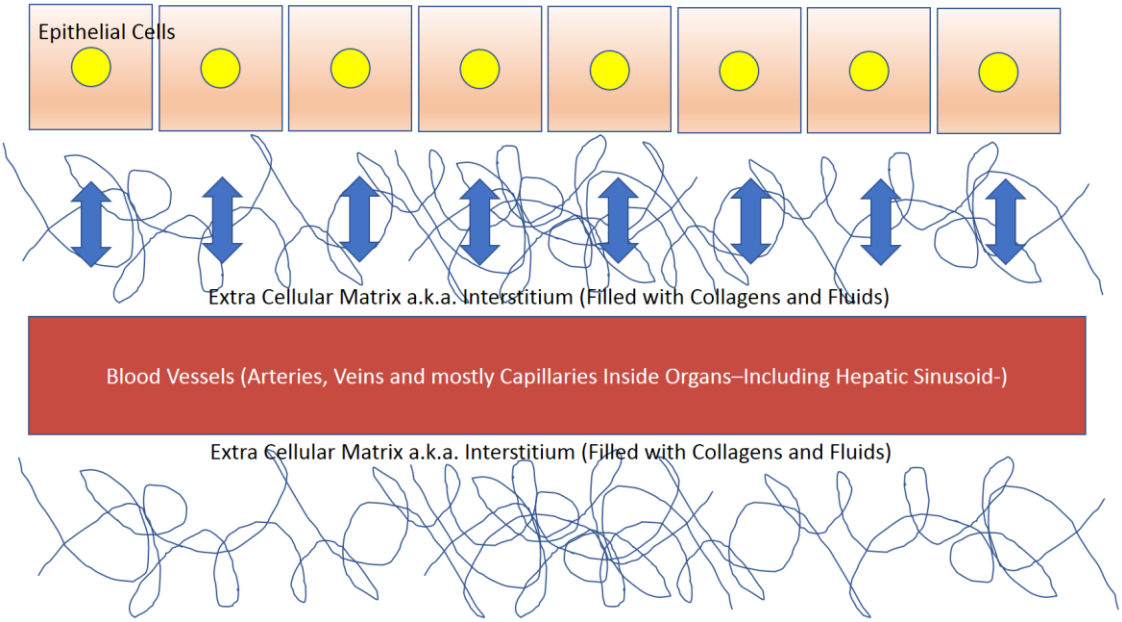

Figure 5 1 Blood/ECM/Organ compartments

Before going on to organ models and metabolism, how the blood- organs/tissues/cells macronutrient/hormone transport is done will be shown. Since metabolites/signal molecule/transcription factors are inside the cells of organs/tissues, they are not part of this chapter's discussion. Arterial blood enters organs/tissues and venous blood exits organs/tissues. In between the two regimes of blood, there is a cluster of organ cells, capillary and extracellular matrix (ECM). In many organ/tissue/cell models, including this research's, the 'cluster' disregards ECM, for convenience and it does not cause big errors and most macronutrients in ECM have the same concentration to either capillaries or organ cells in different organs/tissues. The capillaries inside the 'cluster' carry hormones and macronutrients which will be exchanged/react with

between capillaries and organs' cells (epithelial cell is the surface cells of the organ). These organ cells' and capillaries altogether are 'organs', to be specific, in this model and others, for practical purposes. The concentration of macronutrients inside capillaries are thought to be in thermodynamic equilibrium with organ cells at homeostasis. That is, the homeostatic concentration of macronutrients in organ cells and capillaries would have constant concentrations. Both capillaries and either arterial or venous concentration are assumed to have same concentration for simplicity. Postulating that capillary and either arterial/venous concentrations are the same is accomplished by assuming tissue to be ideal CSTR (Constantly Stirred Tank Reactor, sudden concentration change) rather than ideal PFR (Plug Flow Reactor, axial concentration difference). Otherwise, it will be necessary to consider varying concentration inside capillaries spanning from the entrance (arterial) to exit (venous) side of organs/tissues. It is true that arterial and venous concentration change alongside the axial direction would not matter due to convection where capillaries would have less effect in terms of convection, i.e. diffusion and reaction would matter more; however, for both simplicity and insignificant errors, one can assume the CSTR condition in capillaries (and in fact, organ cells as well). This research, however, further simplifies the 'cluster'. The capillaries and the organ cells are altogether forming hypothetical organ compartment. This, however, can cause confusion in what concentration of macronutrient one should use caused by thermodynamic equilibrium.

To summarize, one can simplify 'Arterial Blood → Capillaries, ECM, Organ Cells → Venous Blood' to 'Arterial Blood → Capillaries, Organ Cells → Venous Blood' to 'Arterial Blood → Hypothetical Organ → Venous Blood'. In this model, capillaries and organ cells without ECM taken into account. This way, this research can differentiate different concentrations in organ cells and capillaries without ambiguity of the concept of 'hypothetical organ'.

6. DIFFERENT ORGANS/TISSUES/CELLS (BRAIN, LIVER, MUSCLE, ADIPOSE TISSUES, PANCREAS, ERYTHROCYTE) AND BLOOD CIRCULATION FOR WHOLE-BODY MODELING

	Rate (μmol/sec, 70kg)
Hepatic Glucose Production	14.35185185
Total Glucose Uptake	14.35185185
Brain	6.481481481
Peripheral (Muscle and Adipose Tissues)	3.240740741
Liver	1.851851852
Gut	1.851851852
Erythrocytes	0.925925926

Table 6 1 Different organs' glucose uptake and release rates

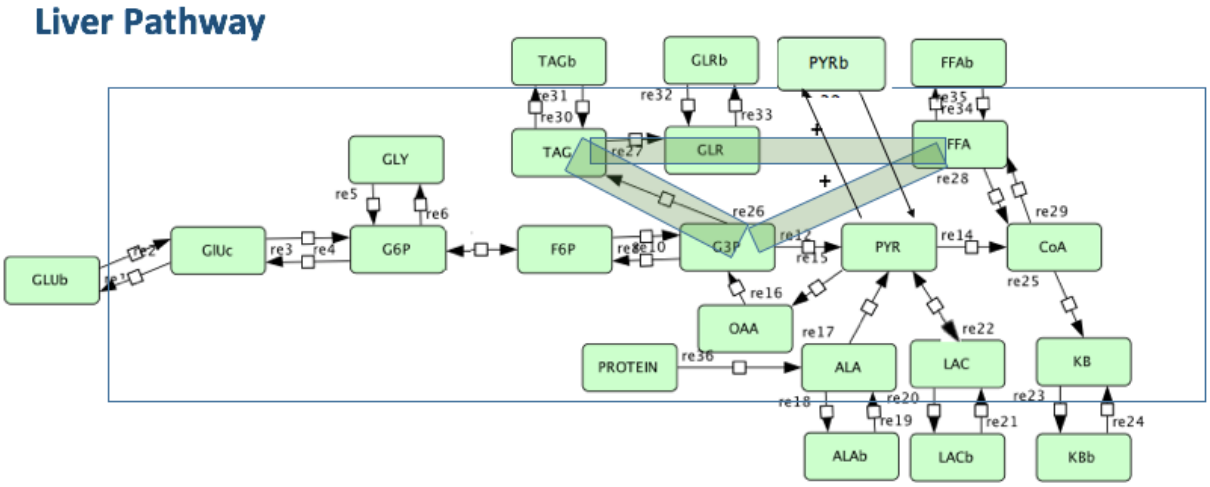

Figure 6.1 1 Liver metabolic pathway

6.1 Liver

Macronutrients	% of Gluconeogenesis	% of Total Hepatic Glucose Production
Lactate	54	13
Pyruvate	4	1
Glycerol	13	4
Amino Acids (Alanine)	29	7
Total	100	25

Table 6.1 1 Liver macronutrients and their contribution in glucose production

Hepatocytes have perhaps the most interesting characteristics of all the organs involved in T2D and many other diseases. The liver is responsible for metabolic elimination in contrast to excretion that occurs in the kidneys. Many metabolites, internal and external chemicals are absorbed and metabolized (a.k.a. detoxification) in the liver's hepatocytes. The end product released from or stored in liver for later use are more stable, less reactive and less toxic. Based on liver metabolism, it is needless to say how much of a central role the liver plays in both healthy and T2D patients under different 'conditions' (Hetherington, J 2011). What kind of unique reactions are taking place in the liver are explained as specific as possible. In all cases, for each organ, unless stated otherwise, macronutrient and metabolite 'level' reactions under different 'conditions' are analyzed.

Let us first start with macronutrients: glucose, glycerol, fatty acid, amino acid, ketone bodies, lactate and TAG.

Glucose plays a big role in liver metabolism in that it releases and absorbs glucose. Hepatic cells, like most of the other cells, use glucose as their primary source of fuel. Liver only absorbs and utilizes glucose under postprandial conditions for storage (glycogen and TAG) and energy (ATP) generation. These absorption and utilization processes can be divided into multiple reactions. The liver releases glucose when the body demands it such as during periods of fasting and/or exercise. Moreover, glucose is needed because some types of cells require them all the time unless alternative fuel source is available. Brain cells use glucose normally but switch to KB during fasting. Erythrocytes, on the other hand, can only use glucose due to lack of mitochondria. Details are laid out in the following.

<Glucose Utilization >The first glucose utilization process (glucose→G6P→F6P››G3P››pyr) is called glycolysis (glucose breakdown) where '→' represents a direct reaction and '››' represents a lumped reaction. This is an anaerobic reaction, generating 2 net ATPs. The (G3P››glycogen) process is called glycogenesis (glycogen generation). Postprandially, absorbed glucose is converted to glycogen to be stored for later use. Glycogen is a polysaccharide and there is a cap or limit on how much glycogen. This limit arises for two reasons: growth/degradation kinetics and limited amount of central protein called glycogenin. Pyruvate having been produced via glycolysis can convert to oxaloacetate (OAA) and acetyl coA (AcCoA), where OAA and AcCoA would enter TCA cycle (OAA+AcCoA››OAA+ATP, stoichiometry disregarded) to produce 34 ATPs under aerobic condition. Pyruvate is not glucose and TCA is not part of glycolysis, an anaerobic process. However, when glucose is utilized to produce energy and produces pyruvate as an intermediate product, it is reasonable to put the TCA cycle as a part of the glucose utilization schematic; it is acknowledged that there can be different perspectives on this. Lastly, glucose can be stored as fat through de novo lipogenesis. This is another form of energy storage besides storage as glycogen. Glucose has multiple 'choke points' where building blocks for TAG assemblage can be produced: FFA produced by AcCoA and G3P produced by glycolysis and glycerol. This is one of the two major ways of producing FFA. The other one is TAG breakdown, which will be explained soon.

<Glucose Production> Many publications and textbooks in biochemistry have overly simplified descriptions of glucose utilization and production. Clear explanations on multiple reactions are necessary, especially forward and backward reaction which deal with multiple overlapping metabolites but with somewhat different reactions. For example, to say that gluconeogenesis is the opposite of glycolysis is not entirely correct. Glucose is produced primarily by one of two mechanisms: glycogenolysis and gluconeogenesis. In case of exercise (either aerobic or anaerobic),

fasting or even starvation, the liver will produce and release glucose by glycogen breaking down into G6P, called glycogenolysis (glycogen breakdown), and G6P converting to glucose. However, one should bear in mind that in both experiments and flux balance analysis (FBA), it is known that G6P from glycogen can go through either glucose production (gluconeogenesis) or F6P production (glycolysis) at any time; depending on mass action, thermodynamics, energy requirement etc., and this is true for any of the metabolites unless a reaction in certain pathway is inhibited by certain mechanisms (e.g. allosteric inhibition). Glucose can be produced via a process called gluconeogenesis (new glucose generation) as well, utilizing mainly four energy sources: AA, lactate, pyruvate and glycerol. The release and absorption of AA, lactate, pyruvate and glycerol in different 'conditions' in liver is discussed in more detail soon. Gluconeogenesis using AA, lactate and pyruvate starts from pyruvate while gluconeogenesis using glycerol starts from G3P. Gluconeogenesis is a unique reaction taking place mostly in liver and under certain circumstance (e.g. starvation), in kidneys. For AA, to simplify assumptions so AA is assumed to be alanine amongst 20 AAs. There are AAs which are gluconeogenic such as alanine, ketogenic or both. For lactate, when one is exercising so that muscle releases extra lactate or there is excess lactate, probably due to fasting, liver absorbs lactate to convert it to pyruvate and then to glucose via gluconeogenesis. The thermodynamic equilibrium between pyruvate and lactate exists at all times. Pyruvate is, as explained, an intermediate product of glycolysis and converted metabolite from AA or lactate, which can convert to either OAA or AcCoA. Conversion to OAA gives rise to G3P/PEP (phosphoenolpyruvate) so that gluconeogenesis can happen. Glycerol can be absorbed or released from liver. When it is absorbed due to diet (postprandial), it can be packaged into TAG. When absorbed during fasting or exercise (TAG stored in WAT can be broken down to release 3 FFA: 1

glycerol for energy expenditure to keep energy homeostasis), it can convert to G3P and undergo gluconeogenesis.

FFA/Glycerol/TAG are grouped together because they are all part of 'fat', specifically TAG metabolism. FFA, glycerol and TAG are to be explained later. Let us quickly discuss the fasting state. The timing of when the body enters fasting state and how to define the fasting state can be different. For example, there are intermittent fasting (skipping a meal each day), long term fasting (skipping meals for days), overnight fasting (glycogen depletion during sleep) or specific time fasting (24 hr fasting etc.) etc. In this model, it is defined to be when both glycogen and gluconeogenesis both become 'prominent' sources of glucose. Relevant simulation will be shown in chapter 13.

<FFA Utilization and Production> FFA is important for many organs/tissues/cells especially during fasting and aerobic and low to moderate intensity exercise state as well as glucose. Liver and muscle use FFA mainly during fasting which is released from WAT, while brain and erythrocytes rely on glucose. Many other peripheral organs/tissues/cells and pancreas may use glucose, FFA, KB or others during fasting. When body's condition changes from fasting to exercising or postprandial, the choice of each organ's fuel may change as explained in glucose part for liver and to be explained for others throughout this chapter. Liver utilizing FFA during fasting implies that it needs aerobic TCA ATP generation process at all times. TCA involves β-oxidation of FFA in mitochondrial matrix and electron transfer on the mitochondrial membrane. FFA, once absorbed into liver for utilization during fasting, converts to AcCoA which has one of two choices: either to enter TCA cycle to generate 34 ATPs or to produce KB. The choice is not random but rather depends on mass action, thermodynamic equilibrium and energy expenditure. The reason why liver prefers fatty acid over glucose during fasting probably is due to high energy output (34

ATPs from FFA vs 2 ATPs via glycolysis in addition to glucose being used preferentially by other types). If AcCoA from FFA chooses to go through TCA, there will be multiple intermediate metabolites involved in this cycle. The TCA cycle metabolites are described in the above illustration. The illustration is detailed enough yet simplified (i.e. lumped) and does not fully describe some of the metabolites' extra reactions. OAA or malate, for example, can go into and out of mitochondria but such reactions are not described because they unnecessarily overcomplicate the model. For AcCoA to go through TCA, however, there should be sufficient OAA so that both metabolites can produce citrate; otherwise, TCA cycle will be minimal. The stoppage on TCA might happen when OAA dries up due to OAA being used as gluconeogenesis source during prolonged fasting. In this case, AcCoA may switch/choose to produce ketone bodies. This will be explained in KB part soon (Guo, Y 2009).

Postprandially, the research will assume consumption of high carbohydrate (characterized as sugar and starch rather than complex carbohydrates and fiber) and high fat diet a.k.a. Standard/Typical American Diet or SA. Once food is consumed orally, the food will be digested throughout digestive system and the digested macronutrients will be absorbed, mostly in the small intestine and rest in the large intestine. Afterwards, the absorbed macronutrients will enter the circulation 'system'. The heart will distribute the arterial blood with macronutrients to each organ (including liver) where the macronutrients in capillaries can be transported into and out from between blood and organs/tissues/cells. The venous blood then enters heart again. In this model, that 100% of the macronutrients entering the small intestine are absorbed (bioavailability measuring how much is absorbed) and that glucose has glycemic index (blood sugar level increase in 2 hours measuring how fast it is absorbed) of 100 (pure glucose) are assumed. When one consumes pure fat (FFA, glycerol, TAG, cholesterol, chylomicron etc.) for a meal, liver consumes fat to produce TAG in

addition to producing ATPs. In other words, consumed extra FFA will be absorbed into hepatocyte for TAG assembly and energy production. This newly made TAGs will later be delivered to WAT (Beller, Mathias 2010).

<Glycerol Utilization and Production> Glycerol is one of the components of TAG breakdown. When a TAG breaks down, it releases one glycerol and 3 FFAs. However, when TAG is composed, glycerol must first convert to G3P and then G3P and 3 FFAs will combine to form a TAG. Glycerol is one of the four gluconeogenic sources and precursor of pyruvate as shown in Figure XXXX. When one consumes fat containing food, the liver will absorb fat including glycerol for TAG composition (after conversion). Here is a recap. During fasting and exercise, alongside FFA absorption, glycerol is absorbed and mostly used as gluconeogenic source and/or pyruvate precursor after conversion to G3P. Postprandially, assuming fat containing food diet, glycerol will still be absorbed to be packed into TAG, after conversion to G3P. TAG absorbed and broken down in the liver can also contribute to glycerol (Beller, Mathias 2010).

<TAG Utilization and Production> During fasting and exercise, TAG from WAT can be released from the liver to provide FFA and glycerol. Postprandially, TAG can be absorbed into and/or packaged inside the hepatic cell and be delivered via lipoproteins to adipose tissue for fat storage or to the rest of the organs/tissues/cells in a human body to provide nourishment. TAG, however, is highly hydrophobic so it is transported, along with cholesterol, via lipoproteins after being secreted from the liver. These lipoproteins are micelles that have proteins and phospholipids on the surface. Depending on the composition (mole %) of lipoprotein, cholesterol, cholesterol esters and TAG in micelle, the micelle can be labelled as High/Medium/Low/Very-Low Density Lipoprotein micelle (HDL/MDL/LDL/VLDL). One should be mindful that all of these micelle

types have TAG inside and measurement of TAG is to measure the concentration/amount of micelles and deduce TAG amount (Beller, Mathias 2010).

Ketone Bodies: Ketone Bodies are a product of AcCoA's partial oxidation. Acetoacetate and β-hydroxybutyrate are the most common KBs in many studies. KBs are used as an alternative fuel for glucose and/or FFA during conditions of low glucose in most cells in the body except erythrocyte, brain and liver. Erythrocytes must use glucose because erythrocytes do not have mitochondria so that FFA cannot be used (β oxidation). Brain cannot use FFA due to the slow rate of FFA transport across the BBB. The liver can use both glucose and FFA but does not use KB due to lack of enzymes which can metabolize KBs. Muscle can use glucose, FFA and KB. The remainder of the organs/tissues/cells of the body may use glucose, FFA and KB.

<KB Utilization and Production> During fasting and exercise, KBs are produced to compensate for body's reduced glucose level and to provide an energy source for the organs/tissues/cells which cannot use FFA. During fasting, it is known that in the order of glucose/FFA – KB – AA, the different organs/tissues/cells use the macronutrients as fasting continues. It is of importance, however, to mention two points: first, there are organs/tissues/cells that only can use specific few macronutrients only; second, different organs/tissues/cells can preferentially use different macronutrients at the same fasting stage. Erythrocytes, brain and adipose tissues are examples of the first point. These organ/tissue/cell types cannot use non-glucose source even during fasting, starvation, exercise or post-feeding. Erythrocytes only uses glucose because they cannot use FFA, KB or AA because the erythrocytes do not have mitochondria. Brain cells can switch from glucose to KB as fasting prolongs; it cannot use FFA or AA because such macronutrients are too big or polar etc. so that they are not be transported across the BBB. Adipose tissues use glucose and FFA.

As mentioned above, muscle, liver and many peripheral organs/tissues/cells may have different KB metabolism. Liver cannot use KB so it should have been in the first point. However, liver deserves a special treatment due to its unique ability to produce KB. Liver can use glucose, FFA and AA for energy source for metabolism and convert metabolites to feed other organs/tissues/cells via gluconeogenesis, ketogenesis etc. KBs produced by the liver for the organs/tissues/cells where they can be used during fasting or exercise. The brain is, however, only able to use either glucose or KB, although KB will be preferentially used in case of fasting. Muscle can use all of the macronutrients: glucose, FFA, KB and AA. It will use the listed macronutrients in the preferential order; mostly KB during fasting and AA during starvation. AA from muscle is released to be delivered to liver for gluconeogenesis as well.

In short, during fasting, the brain will switch from glucose to KB, preferentially. Muscle will switch from glucose/FFA to KB to AA preferentially. Erythrocyte must always use glucose. Adipose tissues use glucose/FFA and release FFA. Liver must release glucose and KB for the organ/tissue/cell types mentioned here while using glucose/FFA. Different organs/tissues/cells, of course, can use glucose during fasting but preferentially choose alternative energy fuel so that glucose can be spared for erythrocytes. Postprandially, KBs can be made when one is in early stage of Atkins diet and/or ketogenic diet. Prolonged ketogenic diet (high fat, low carbohydrate) will use up all the glycogen from the liver and fat will be the most prominent energy fuel for liver and other organs/tissues/cells except erythrocyte. Excess fat source can convert to AcCoA, which will in turn convert to KB. Again, this KB can fuel brain and other KB using organs/tissues/cells just like during fasting and exercise.

Lactate is another source of gluconeogenesis for liver.

<Lactate Utilization and Production> Liver absorbs lactate mainly for gluconeogenesis during exercise and fasting. During exercise, depending on duration and intensity, two core factors of exercise, the muscle will choose to release lactate. Since muscle cannot utilize lactate for energy, the liver must do so by using lactate for its own energy fuel and/or by converting lactate to glucose for muscle and other organs/tissues/cells. Postprandially, lactate could be absorbed for TCA and hepatic metabolism.

AA There are 20 AAs and alanine is considered for simplicity. As shown above, some AAs can be ketogenic and some for gluconeogenic. Alanine is a gluconeogenic AA.

<AA Utilization and Production> During exercise and prolonged fasting (starvation), AA can be provided to muscle for gluconeogenesis. Approximately 20% of the muscle is protein. Rest are mostly water and other macronutrients. Proteins in muscle are myosin, actin etc. Once the body enters prolonged fasting state or perhaps certain types of exercise, the muscle protein can be degraded as the final source of fuel for the body. KBs will be produced by liver and used by certain organs/tissues/cells preferentially before AA is used by liver for gluconeogenesis. This shows why when one proceeds a prolonged fasting, the muscle loses mass and debilitates. The AA absorbed by the liver can be used as gluconeogenic source (and ketogenic for certain AAs). After the body goes beyond this stage, one may die of hunger.

Glycogen It is one of the energy storing metabolites. Glycogen, as previously explained earlier, has a central protein called glycogenin where polymer can grow from. The capacity on glycogen storage in liver and muscle is known. Glycogen at its fullest storage in both organs can be up to a kilogram each. Muscle glycogen will be explained in detail in the muscle section.

For liver, glycogen cannot be stored more than certain amount due to two major reasons: first, kinetics of glycogen growth and degradation and second, the limited amount of glycogenins. In many published models, glycogen biophysics is not considered much. That is, the second reason is usually omitted.

The first reason alone is not able to fully explain how glycogen behaves. In many models, glycogen is simply stored and used depending on consumed glucose concentration and glycogen's glucose 'unit' concentration. Therefore, the tug of war between two concentrations is achieved due to concentration on input and output of glucose. This is partially true. The glucose can be linked to glycogen polysaccharide chain due to glucose concentration but glycogen does not just degrade due to how much glucose monomers there are. In fact, it is important to think of what concentration of polymer means. Polymer's property can be described as molecular weight and how many units of monomers there are etc. rather than concentration. Also, what is driving polymer's degradation would be temperature, acidity etc. but concentration of monomer units in laboratory environments. Also, using glycogen's glucose unit concentration can cause parameter errors in building models. Therefore, it would be wrong to say that degradation of glycogen is due to glycogen's or glycogen glucose 'unit' concentration. Therefore, 'decoupling of energy input and output' idea is introduced. It is thought that what is driving energy expenditure, in other words, degradation of energy storage such as glycogen and TAG is not the concentration of the polymer or polymer's unit concentration but energy requirement by a human body (Müller, C 1997). The exact signal processes on how the energy demand of a human body will cause glycogen to degrade is not known but by decoupling energy expenditure (glycogenolysis) from the energy input (glycogenesis), this model can be made more robust and less stiff. This is also physiologically correct because the purpose of glycogen and TAG is to store energy in case of a human body needs to expend energy. Therefore,

glycogenesis is glucose concentration dependent first order kinetics while glycogenolysis is energy expenditure dependent zeroth order kinetics in this model (Lomako, J 2004).

The energy expenditure is mainly composed of three factors: BMR, thermogenesis from indigestion and keep bodily temperature homeostasis and exercise. BMR is the minimum energy used by body to survive such as energy used for enzymatic metabolism, cellular network, heart palpitation etc. BMR is variable in individuals of different genders, age, nutritional condition, RQ (respiratory quotient) etc. The some of the most important factors of BMR are muscle mass, gender and age. Each organ will need to spend certain amount of energy in homeostasis for survival. Thermogenesis is extra energy expenditure on top of BMR to keep thermal homeostasis and to digest food. Exercise is another extra energy expenditure which is dependent on duration and intensity (Oldfors, Anders 2017). This will be explained in muscle section. Further explanations on RQ and a summary of macronutrient usage during different 'conditions' plus additional conditions will be explicated soon.

Secondly, central proteins called glycogenins play a big role in glycogenesis. Glycogenins build glycogen once glucose is consumed. Glycogen only starts to be formed and accumulated on clean glycogenins which are unoccupied by glycogen. This is helpful in understanding the metabolism of glycogen in that it shows that glycogen can be made only under certain circumstances. On the other hand, glycogen can be degraded from any glycogenins occupied by glycogen. The degradation process is rather obvious. This poses an interesting question on why the glycogen building process must start from clean slate glycogenins and why there are only limited number of glycogenins. These are important queries especially given that the limited amount of glycogenins and kinetics elaborated above give rise to several balls of glycogen reaching its storage capacity in liver and muscle in scanned images. Why clean glycogenins are the starting point for

glycogenesis are not unknown but one could guess why there are certain number of glycogenins. It is thought that homeostasis in a human body will keep glycogenins at certain amount by degrading excess glycogenins so that AA remnants can be reused for different purposes; or one could think that there are excess undegradable glycogenins but many of them are sequestered somehow so that only certain number of glycogenins can be seen. This sequestration idea is inspired from ER's calcium sequestration/release mechanism (Marchand, I 2002).

RQ is a measurement of a ratio of oxygen (O2) and carbon dioxide (CO2) from organs/tissues/cells. Every macronutrient going through TCA cycle will use O2 and release CO2; however, different macronutrients will use different amount of O2 and release different amount of CO2. For example, carbohydrate will undergo 'C6H12O6 + 6 O2 → 6 CO2 + 6 H2O'. The corresponding 'R.Q. = 6 CO2 / 6 O2 = 1.0' can be calculated. On the other hand, palmitate (fatty acid) goes through 'C16H32O2 + 23 O2 → 16 CO2 + 16 H2O' and has 'R.Q. = 16 CO2 / 23 O2 = 0.696'. When observing what kind of nutrients one consumes, RQ in exhalant breath can help showing the energy fuel usage. Since bodies can use multiple nutrients at the same time, RQ is only representative of average of whole situations, but still can be a good noninvasive measurement. Likewise, when the arterial and venous O2 and CO2 level are measured for different organs, one can think of approximately what kind of nutrients are preferred and used under different 'conditions' for each organ (Kim, Jaeyeon 2006).

<Glycogen Utilization and Production> During exercise and fasting, glycogen can be used for supplying glucose until it runs out. During exercise and fasting, glycogen will break down to supply glucose. While postprandially, both muscle and liver glycogen will get bigger. Liver can store up to 500g of glycogen while muscle can store up to 100g (Browning, Jeffrey D 2011).

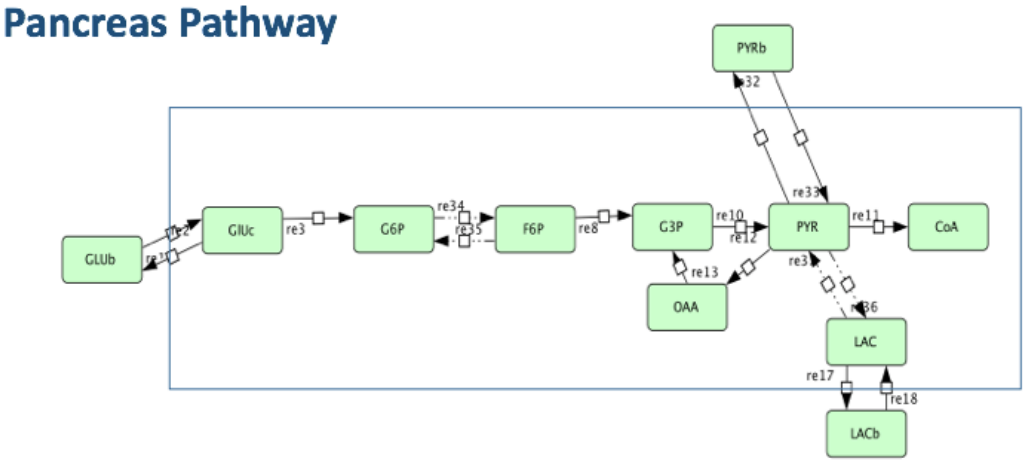

Figure 6.2 1 Pancreas metabolic pathway

6.2 Pancreas

Here are some information on pancreas. The pancreatic acini are clusters of cells that produce digestive enzymes and secretions and make up the bulk of the pancreas. The endocrine function of the pancreas helps maintain blood glucose levels, and the structures involved are known as the pancreatic islets, or the islets of Langerhans. 1 million islets in a healthy, adult pancreas. They make up only 1 to 2 percent of the entire organ. Within each islet are several types of cells, which work together to regulate blood sugar. α (15-20%), β (65-80%), δ (3-10%), γ (3-5%), ε (1%). The feedback system of the pancreatic islets is paracrine, and is based on the activation and inhibition of the islet cells by the endocrine hormones produced in the islets. 70% β cell,20% α cell. α cell is probably responsible for glucose-induced pancreas lactate release while β cell lactate release rate almost remain the same over the whole concentration range of glucose In blood. For the enzyme controls reaction pyr --> oxa, it is highly expressed in β cell compared to α cell (Matthews, D. R 1985).

Glucose (mM)	Insulin Release Rate (nmol/sec, pancreas)
0	0.02
1.8	0.02
2.8	0.03
3.5	0.03
4.3	0.06
5.5	0.12
6.7	0.22
8.4	0.30
11.1	0.38
16.8	0.59
20	0.58
28	0.63

Concentration (mM)	Insulin Secretion Rate Fold Change (β cell)
Glucose	
2.8	1.16
5.6	1.44
8.4	2.07
11.2	2.11
16.7	2.45
Pyruvate	
2	2.08
20	2.3
Lactate	
2	1.24
20	1.67

Table 6.2 1 & Table 6.2 2 Insulin release rate change at different macronutrient concentrations

7. RELEVANT DATA

Here is science of fasting and weight loss. Ghrelin-hunger hormone. HGH-sparing protein, mobilizing FA. Insulin-dependent lipolysis. Ketone Bodies-fasting. Leucine-protein synthesis. When low carb diet: metabolism down, muscle loss. When fasting: metabolism up, fat loss. Resting energy expenditure increases in early starvation, accompanied by an increase in plasma norepinephrine. This increase in norepinephrine seems to be due to a decline in serum glucose and may be the initial signal for metabolic changes in early starvation.

Energy requirement per day is calculated in this research. 1TAG -> 3FA + Glycerol is assumed. Assuming that a human body needs fat as the only external food source for daily BMR, the following calculation can be done. BMR: 1500 [kcal/day], FFA energy content: 9 [kcal/g], palmitic molecular weight: 256.42 [g/mol]. A daily BMR FFA uptake requirement is assumed 1500/9 [g/day] = 166 [g/day]. Then one can change the aforementioned requirement to moles for ratio comparison: 1500/9/256.42 [mol-FA/day]. Assuming 2 glycerols become 1 glucose (GNG), TAG breakdown can be thought to produce 6FA and 1 glucose. 1500/9/256.42*(1/3)*(1/2) [mol-glucose/day]. By reverting to weight scale for understanding: 180 [g-glucose/mol]* 1500/9/256.42*(1/3)*(1/2) = 19[g-glucose/day], can be obtained. As a daily glucose requirement by organs/tissues/cells, especially for erythrocytes is 14.4 [g/day], maybe rest of the glucose is used for other organs/tissues/cells. The remaining glucose can be possible cause of dawn phenomena in IR patients.

8. FATTY ACID TYPES AND METABOLISM

NAFLD (non-alcoholic fatty liver disease). Serum FFA profiles of NAFLD patients were significantly higher compared with HC, and obese NAFLD patients presented the poorest FFA profiles. Furthermore, 14:0 (myristic acid) and 16:1 (palmitoleic acid) are of promising diagnostic value in the early diagnosis of NAFLD especially among normal weight individuals.

Elevated serum 16:1 profile is also significantly related to abdominal adiposity and fatty liver disease (FLD). A rich source of 14:0 has been reported to acutely raise LDL levels. Beneficial effect of Mediterranean diet in improving cardio-metabolic health and plummeting cardiovascular related morbidities and mortalities has been reported. (Presumably) Down-regulating the action of 7α- hydroxylase (a rate limiting enzyme in bile synthesis and cholesterol regulation), thereby increasing fecal excretion of cholesterol. Both 14:0 and 16:1 can be derived from diets and whether their dietary intakes were associated with serum levels were not studied (Bolinder, J 2000).

Here are lipoprotein miscelle studies regarding consumption of nutrients: Higher intakes of saturated fatty acids leads to higher LDL cholesterol, higher HDL cholesterol, lower triglycerides, lower total cholesterol-to-HDL cholesterol ratio. Whereas increased carbohydrate intake causes lower LDL cholesterol, lower HDL cholesterol, higher triglycerides, higher total cholesterol-to-HDL cholesterol ratio (Bolinder, J 2000).

Diet	Total Calories/Day	Carbohydrate Grams/Day (cal %)	Protein Grams/Day (cal%)	Fat Grams/Day (cal %)
Standard American Diet (SAD)	2200	275 (50)	82.5 (15)	85 (35)
Paleolithic Diet	1388	129 (39)	92 (27)	46 (28)
Mediterranean Diet	1823	211 (47)	88 (20)	59 (28)
Ketogenic Diet (Similar to Early Atkins Diet, Low Carb)	Variable	13 (5)	52 (20)	87 (75)

Table 8 1 Different diets' macronutrient composition

	14:0	16:1	Total Fat
BMI	+	+	+
Body Fat	+	+	+
Fasting Insulin	+	+	+
TAG	+	+	+
HDL	NS	NS	NS
LDL	+	+	+

Table 8 2 Different fatty acid types and associated biomarkers

9. GLUCOSE AND FFA CORRELATION WITH MORBIDITY AND CARDIAC HEALTH

The research will introduce some literature where 'what is believed to be true of a relationship between certain macronutrient and certain disease' contradicts to 'what the real correlation is'. These revelations are instrumental and insightful in understanding T2D and many other diseases of interest because they tell us that one should aim for finding out the root cause rather than superficial correlations. In Lancet paper, there was a study on total mortality and major cardiovascular disease depending on consumption of two types of energy source/nutrients: fat and carbohydrate. In 'Food Pyramid' and many of the worldwide food guidelines say that high carbohydrate diet is more desirable so that cutting down fat's content in food as much as possible would be good for preventing diseases such as stroke, atherosclerosis, cardiac arrest, high cholesterol, high pressure etc. According to 'Big Fat Surprise', the high carbohydrate diet and high carbohydrate based Food Pyramid has not done a good job in reducing mortality, T2D and cardiovascular diseases. It says that Mediterranean diet (berries and nuts) did not improve nor exacerbate the mortality, T2D and cardiovascular diseases. On the other hand, Atkins and ketogenic diet (low carbohydrate and high fat) reduce T2D and cardiovascular disease risk. The book concludes that dietary fat consumption does not lead to cardiovascular diseases and having less carbohydrate helps to reduce the risk. Likewise, the Lancet paper concludes from its meta-studies that any kinds of fat including saturated and mono/polyunsaturated fat are positively correlated with decreasing both mortality and cardiovascular disease risk while carbohydrate is negatively correlated. Both the book and the article indicate that if one is to consume same amount of calorie for everyday activity (BMR + digestion + optional exercise), reducing the percentage of carbohydrate contribution of energy % (not weight %) is good for health. The increase in fat energy

contribution % is not only enough for survival (to live) but also maintaining health without major health risks (to live healthy).

This is counterintuitive to a widely held belief that high fat related diseases such as high cholesterol (non-HDL) and atherosclerosis. According to Big Fat Surprise, to treat high cholesterol, people used drugs to lower the cholesterol level regardless of density; this brought about declining health for women and children. Atherosclerosis has been thought to be caused by fat consumption due to the white sticky plague narrowing and hardening the blood vessel without fully understanding the mechanism. T2D, likewise, has been blaming obesity as the main reason of T2D progression. In this case visceral fat depot around organs, not dietary fat nor high BMI due to subcutaneous fat, may be responsible for the onset.

In a nutshell, it is needed to figure out the causation not correlation when it comes to disease. Shown are the examples of T2D, high cholesterol and atherosclerosis (Beller, Mathias 2010).

10. EFFECTS OF HORMONES

There are different insulin increase effects on glucose.

1. Insulin sensitive organs/tissues/cells by direct action.

This increases glucose transport in muscle, adipose tissues but not in liver. Muscle and adipose tissues glucose transport = non-equilibrium (kinetic). liver glucose transport = near equilibrium (thermodynamic). Muscle and adipose tissues deploy more GLUT4 onto the surface (perhaps by SNARE).

Increases glycolysis by increasing activities of HK (to G6P), 6PFK (to F6P) in muscle and adipose tissues. HK by perhaps by signal pathway, 6PFK perhaps by signal pathway + F-2,6-P. Increases glycogenesis in adipose tissues (direct), muscle and liver (direct? + indirect). If glycogen in adipose tissues is replete, excessive carbon is stored as lactate and delivered to liver for indirect glycogenesis. Glycogenolysis may be more sensitive than glycogenesis (Marchand, I 2002).

2. Insulin sensitive organs/tissues/cells by indirect action.

Mobilization of adipose tissues affects intestines and brain metabolism indirectly.

There is insulin increase effects on FA. Decreases FA lipolysis in adipose tissues, hence, lower plasma FA. Increases FA and TAG synthesis in T. Increases VLDL synthesis in liver. Increases TAG uptake in muscle and adipose tissues. Decreases HSL (TAG breakdown) activity and increases LPL activity (TAG absorption) + plus.

There is insulin increase effects on glucose and FA. β oxidation of FA inhibits glucose transport and glycolysis. If insulin increases, HSL activity is inhibited as a result of cAMP activity decrease (signal pathway) in adipose tissues, plasma FA decreases and less FA into muscle at the same time

when malonyl-CoA inhibits FA oxidation. HSL activity decreases in adipose tissues so Less FA into muscle and Malonyl-CoA inhibits β oxidation. Here is the reaction of malonyl-CoA production. AcCoA + ATP + CO2 -> malonyl-CoA + ADP + Pi.

However, now it is suggested that it may not be β oxidation that inhibits glucose transport but excess FA in muscle may interfere with signal pathway of insulin (PI3K activity decreases as a result of IRS-1 interference), to be explained. Lipotoxicity in pathogenesis of T2D and glucose intolerance. HSL and LPL becoming less sensitive to insulin may cause lipotoxicity. Perhaps this may lead to excess FA in muscle. In the end, higher organ/tissue/cell and plasma FA will impact muscle, liver and pancreas.

Blood flow increases as a result of endothelium derived nitric oxide (perhaps glucose transport increases as well). It can be secreted by cholinergic (vagus nerve stimulation) when fed, a non-nutrient secretagogue. AA promotes both insulin (proteogenesis) and glucagon.

Acute exercise may lead to more GLUT 4 on sarcolemmal membrane, while chronic exercise may lead to higher GLUT 4 mRNA expression, higher insulin sensitive signaling (talk of mechanistic, hormonal, nervous). Also, glycemic index (GI) for different carbohydrates may be important for insulin secretion profiles.

Carbohydrate metabolism: It increases the rate of glucose transport across the cell membrane in muscle and adipose tissue. It increases the rate of glycolysis in muscle and adipose tissue by stimulating hexokinase and 6-phosphofructokinase activity. It stimulates the rate of glycogen synthesis in a number of organs/tissues, including muscle, adipose tissue and liver. It also decreases the rate of glycogen breakdown in muscle and liver. It inhibits the rate of glycogenolysis and gluconeogenesis in the liver.

Lipid metabolism: It decreases the rate of lipolysis in adipose tissue and hence lowers the plasma fatty acid level. It stimulates fatty acid and triacylglycerol synthesis in organs/tissues/cells. It increases the rate of very-low-density lipoprotein formation in the liver. It increases the uptake of triglyceride from the blood into adipose tissue and muscle. It decreases the rate of fatty acid oxidation in muscle and liver. It increases the rate of cholesterol synthesis in the liver.

Protein metabolism: It increases the rate of transport of some amino acids into organs/tissues/cells. It increases the rate of protein synthesis in muscle, adipose tissue, liver and other organs/tissues/cells. It decreases the rate of protein degradation in muscle. (and perhaps other organs/tissues/cells). It decreases the rate of urea formation.

There are glucagon effects on glucose as well.

Glucagon signals that blood glucose concentration is low and organs/tissues/cells respond by mobilizing stored energy via glycogenolysis, mobilization of FA and gluconeogenesis; inhibits glycogen synthesis and glycolysis and binds to receptors in adipose tissues activating lipase which mobilizes FFA and glycogen

Epinephrine does the following.

When released into blood to prepare the organs/tissues/cells for fight or flight response; binds to receptors that are found primarily found in muscle, adipose tissues and liver. In liver, epinephrine activates gluconeogenesis and glycogen phosphorylase and simultaneously inactivates glycogen synthase by cAMP phosphorylation cascade. Glycogen stores are mobilized increasing blood glucose concentration; promotes glycolysis and FA from adipose tissues.

Cortisol does the following. It is asteroid hormone that signals long term stress such as fear, pain or low blood glucose levels; acts on liver, muscle and adipose tissues; slow acting hormone that

alters metabolism by changing metabolic enzyme gene expression; especially good at stimulating adipose tissues to release FFA from TAG (cortisol passes through the gene for it is lipophilic). It stimulates the breakdown of muscle proteins and increases the rate of export of AA to the liver for gluconeogenesis by increasing pyruvate carboxylase in the liver (Matthews, D. R 1985).

11. DIFFERENCE BETWEEN HEALTHY AND T2D PEOPLE

In this section, the difference between healthy and T2D people is addressed. One of the biggest difference is gluconeogenesis rate. In healthy people, gluconeogenesis rate always is kept constant. However, in T2D people, twofold increase in rates of glucose production attributed to an increased rate of gluconeogenesis.

When characterizing T2D, one normally thinks of insulin resistance (IR). The definition, mechanism and characteristics of IR is explained in the next section. In this research, it is thought that sugar production being another characteristic of T2D, in addition to IR. T2D revolving around IR concept says that glucose level is high due to incapability of normal pancreas to release excessive insulin to compensate for hyperglycemia. IR people who can secrete excessive insulin to compensate for hyperglycemia will be NGT (Normal Glucose Tolerant) people, not T2D. However, given the facts that T2D people experience dawn phenomena, one also needs to think about sugar production being another characteristic of T2D. Dawn phenomena is defined as overproduction of glucose between the midnight and 8am (overnight sleep). This raises blood glucose level in T2D and some healthy people without food consumption without apparent reasons. The glucose level decreases to T2D baseline once breakfast is consumed as insulin is released. This phenomenon cannot be explained by merely IR. Eventually, one can think of following questions. For NGT IR patients, does insulin increase accordingly to glucose level, above normal level (excessively) for compensation? If so, β cells may have very high capacity to compensate for sugar overproduction (proven in in vitro β cell glucose infusion experiment). For T2D people, can normal pancreas release excessive insulin but still has hyperglycemia? If so, T2D patients may have both IR and sugar overproduction, although their contribution may be different in different people (Matthews, D. R 1985).

Here are more facts and comparison regarding healthy and T2D people.

In liver, the following happens. Increased free fatty acid delivery, and reduced VLDL catabolism by insulin resistant adipocytes, results in increased hepatic triglyceride content and VLDL secretion (Hetherington, J 2011). This may lead to NAFLD.

In muscles, the following happens. Muscle develop insulin resistance 10 years before type 2 diabetes kicks in. Muscle glycogen synthase dysfunction is thought to be responsible for IR more than any factors. Gulli et al. (32) were the first to demonstrate that the NGT offspring of two type 2 diabetic parents demonstrated marked muscle insulin resistance but normal sensitivity to the suppressive effect of insulin on hepatic glucose production. However, a normal basal rate of HGP in the face of fasting hyperinsulinemia could be construed to indicate the presence of hepatic insulin resistance. The development of hyperglycemia further stimulates β cell secretion of insulin, and the resultant hyperinsulinemia causes a downregulation of insulin receptor number and of the intracellular events involved in insulin action, thus exacerbating the insulin resistance. In some individuals, the persistent stimulus to the β cell to oversecrete insulin leads to a progressive loss of β cell function. After 72 hr of sustained physiologic hyperinsulinemia, insulin stimulated muscle glycogen synthase activity, total body glucose uptake, and nonoxidative glucose disposal (primarily reflects glycogen synthesis in muscle) were significantly reduced in health people. Taken together, these findings indicate that hyperinsulinemia is not only a compensatory response to insulin resistance, but also a self-perpetuating cause of the defect in muscle insulin action (Matthews, D. R 1985).

Meanwhile for adipose tissues, the following happens. In insulin resistance the effects on adipose tissue are similar, but in the liver the increased free fatty acid flux tends to promote hepatic very low density lipoprotein (VLDL) production whilst ketogenesis typically remains suppressed by

the compensatory hyperinsulinemia. Furthermore, since lipoprotein lipase activity is insulin-dependent and impaired by insulin resistance, peripheral uptake of triglycerides from VLDL is also diminished. These mechanisms contribute to the observed hypertriglyceridemia of insulin resistance. In addition to free fatty acids, adipose tissue secretes a number of cytokines which have systemic effects on insulin resistance. Adipocytes from diabetic and insulin resistant individuals have reduced GLUT 4 translocation, impaired intracellular signalling via reduced IRS-1 gene and protein expression, impaired insulin-stimulated PIP-3 kinase and Akt (protein kinase B) (Sonntag, Annika G 2012).

In general, the following is known. The majority of type 2 diabetic subjects are obese, they also have daylong elevation of the plasma free fatty acid (FFA) concentration and increased circulating levels of inflammatory cytokines. Because elevated plasma glucose, FFA, and cytokine concentrations all can induce insulin resistance, it is extremely difficult to separate the contribution of each of these metabolic defects in the pathogenesis of type 2 diabetes. Initially, it was assumed the mechanism was a competition between fatty acid and glucose oxidation, the so-called Randle cycle, but a much more complex effect of fatty acids on insulin signaling has evolved. One concept is excess fatty acids interfering with insulin signaling via PKC induced serine phosphorylation of IRS-1 with support for that idea that PKC-theta knockout mice are resistant to fat-induced insulin resistance. The elevated fasting plasma FFA concentration in the presence of fasting hyperinsulinemia and the impaired suppression of plasma FFA during the euglycemic insulin clamp. These findings indicate the presence of marked adipocyte resistance to the antilipolytic effect of insulin. Impaired insulin-mediated suppression of whole-body lipid oxidation also was present in the NGT offspring (Bolinder, J 2000).

12. RECAP ON T2D PROGRESSION WITH THOUGHTS ON HIF

Before starting this section, some terms and relationships need to be defined clearly. Lean and obese. NGT (Normal Glucose Tolerant), IGT (Impaired Glucose Tolerant) and T2D. Nondiabetic, prediabetic and diabetic. Insulin resistant (IR) and insulin sensitive. First, lean and obese are determined by several measures including BMR, waistline etc. Second, NGT, IGT and T2D indicate how high the blood glucose level is. NGT people will have normal glucose level while T2D will have fully developed hyperglycemia. IGT people have higher glucose level than NGT people but lower glucose level than T2D. Third, nondiabetic, prediabetic and diabetic are interchangeable terms with NGT, IGT and T2D respectively. Last but not least, insulin resistant means for the same glucose to be absorbed, higher insulin level is needed. Healthy people are usually insulin sensitive (normal insulin level lets glucose to be absorbed without hyperglycemia) (Bolinder, J 2000).

One thing to keep in mind is that prediabetic/T2D people do not necessarily have β cell dysfunction [ref]. β cell still can release more insulin to compensate for higher glucose level up to a certain point in insulin resistant people. Hyperinsulinemia is another feature of prediabetic and T2D. Hyperinsulinemia, in turn, may reduce insulin receptors in different organs/tissues/cells which can further exacerbate glucose intolerance (Sonntag, Annika G 2012). This may cause β cell dysfunction in the final stage, but not necessarily.

Given the information, this research concluded that there are several possibilities of disease states. By categorizing three groups, lean/obese (1st), NGT/IGT/T2D (2nd) and IR/non-IR (3rd), one can hypothesize and test what may happen to people of different health conditions. In one experiment, a group of patients, who were offspring of T2D parents, were lean, NGT and IR. This means, genetically T2D disposed offspring did not have high BMR and had normal glucose level but had

IR. Their pancreas secretes more insulin, as a result, to compensate for IR to keep glucose level desirable. This also corroborates the fact that β cell is not necessarily broken down (Sonntag, Annika G 2012).

Recent research suggests that the possible cause of insulin resistance (IR)/T2D initiates from the muscle. However, there are two competing ideas on which may be the exact starting point: One suggests T2D starting from mitochondria in muscle and the other suggests that it starts from lipotoxicity in myocytes' cytoplasm (Sonntag, Annika G 2012). The explanations regarding these are followed below.

First, an idea that IR/T2D is caused by muscle's mitochondrial genes for prediabetic and T2D people suggest the following. Mitochondrial energy generation related genes are underexpressed causing malfunction in β oxidation in mitochondria. Genes involved in TCA, oxidative phosphorylation and glycolysis are suppressed. β oxidation is a reaction where FFA absorbed into muscle cells producing 8 AcCoA as products, where AcCoA can be used as a substrate for TCA. This leads to less oxidized FFA and then higher lipid level in muscle leading to lipotoxicity. The difference in energy generation between non-prediabetic (genetically not predisposed) and prediabetic (showing IR but not T2D, genetically predisposed) is 90% : 5% (Bolinder, J 2000).

Second, there is an idea that IR/T2D is cause in muscle by lipotoxicity in prediabetic and T2D people. Lipotoxicity causes serine phosphorylation which downregulates tyrosine phosphorylation on IRS-1 alongside PI3-K and Akt somehow being affected as well. Glucose transfer and nonoxidative glucose disposal (i.e. glycogenesis) are downregulated. Plasma glucose and FFA is higher in prediabetic NGT (Normal Glucose Tolerance) IR and T2D people. Such high levels of FFA will be absorbed into muscle more readily. FFA, its derivatives and other types of fat such as palmitoyl carnitine (a product of CAT reaction and precursor for mitochondrial β oxidation) or

DAG (diacylglyceride) may cause disruption in muscle cells. It is thought that lipotoxicity may cause downregulation of mitochondrial energy generation genes in the end. Chronic lipid exposure in healthy people correlated with decline in insulin dependent glucose uptake and tyrosine phosphorylation. It may be due to less GLUT 4 deployment (Bolinder, J 2000).

Here is a recap on signal molecular cascade. Insulin binds to insulin receptor (IR) α portion. Then β IR site undergoes conformational alteration due to autophosphorylation on tyrosine. Then the phosphorylation of IRS (IR substrate) -1 and IRS-2 follow. Then IRS-1 and IRS-2 activate PI3K. Then PI3K catalyzes phosphorylation of membrane phosphoinositides to produce phosphatidylinositol-3,4,5-phosphate. Phosphatidylinositol-3,4,5-phosphate regulates the activity of PDK-1. PDK-1 phosphorylates protein kinase B/Akt at Ser/Thr. Akt activates AS160. AS160 has two significant ways on GLUT4 translocation. First, AS160 reduces the tethering of the GSV by acting on TUG protein (pull), releasing GSV to the periphery. Second, AS160 increases the activity of Rab proteins which will stimulate (push) translocation of GSV. Previously described insulin dependent signal pathway involves microtubles which may be caused by Rab. Then the microtubles approach actin filaments, tossing the GSV. By tethering, the GSV is navigated towards plasma membrane. By docking and fusion by SNARE complex, the GSV is finally fused on the membrane. This last process caused by AS160, in short, translocate GLUT4 onto the membrane by 5-30 folds by following possible mechanisms: GLUT4 GSV exocytosis rate constant increases (maybe Rab), GLUT4 endocytosis rate constant decreases (maybe TUG). Some also suggest that GLUT4 in the cell surface recycling pool increases (due to endosome and Golgi complex). T2D patients tend to have dysfunction in one of the molecular reactions (Bolinder, J 2000).

Combining muscle IR/T2D onset idea and signal molecular cascade, one can conclude the following. Various lipid metabolites, including ceramide, DAG, and long-chain acyl-CoA species,

are believed to induce insulin resistance (48) secondary to activating Ser/Thr kinases (e.g., JNK, mTORC1, IKK) that catalyze site-selective Ser phosphorylation of the insulin receptor and IRS-1, resulting in attenuated insulin signaling via IRS-1/PI3K, Akt, and other key steps (Sonntag, Annika G 2012).

In addition to the aforementioned possible causes of IR/T2D, the following is suggested. It is thought that there is even more fundamental cause of IR/T2D, which arise from visceral adipose tissues. From NAFLD study, it was found out that high levels of FFA (14:0 and 16:1) are relevant to having NAFLD no matter they are lean or obese. When a person has NAFLD, they also tend to have high level of visceral adipose tissues. Also, high level of adipose tissues is known to be associated with T2D. High level of hypertrophic adipose tissues has several characteristics including fibrosis, inflammation, higher FFA release and hypoxia. Hypoxia causes HIF molecules to increase in visceral adipose tissue which increase adipokine secretion, FFA secretion and cause inflammation in adipose tissues. This unique feature is distinguished from subcutaneous fat where hypoxia is not of much issues. The diet which causes 14:0 and 16:1 level to increase are not yet known. It is thought that lower BMR, lack of exercise and diet may cause visceral adipose tissues to build up (Bolinder, J 2000).

13 MATHEMATICAL SETTINGS AND METHODS FOR WHOLE-BODY MODEL

13.1 Introduction

(1) The pathway for specific organ is defined.

Also known as network reconstruction. Each organ has different metabolite profile. How to build metabolite pathway model for different organs and how to connect them will be explained.

(2) The flux balance analysis is conducted.

Flux Balance Analysis (FBA) is a linear algebraic calculation with linear constraints considered. Usually one can set an objective function and adjust reaction fluxes within constraints using linear programming. This can be achieved in many different programming languages.

How to build stoichiometric matrix and how to calculate steady state flux is explained. Why matrix form is informative is discussed.

(3) Detailed kinetic for each enzyme is written down.

Flux is the rate of chemical reaction so for each flux one can rewrite as it a reaction rate equation depending on metabolite's concentration.

The form of equation depends on the enzyme kinetics. Different kinds of enzyme kinetics and cofactor or hormone that will affect the enzyme will be explained.

(4) The simulation with random parameters which satisfy the steady state is performed.

The condition of this model is changed. For instance, the initial condition of the model is changed and whether the model still make sense is observed.

(5) The parameters are optimized.

Here, a strategy to optimize the parameters is developed.

(6) The model is validated and sensitivity analysis is performed.

Once the optimization process is over, validation should follow. The data set used for optimization for parameter estimation must be different from the data set used for validation. If the same data set are used for both optimization and validation, overfitting can happen. Validation is needed to see if the estimated parameters are working properly. If optimized parameters are fairly correct, the error between simulated plots and the real validation data would be minimal. In this model, since there are a plenty of different types of data set, the data sets can be split 50/50 for optimization and validation purposes.

(7) Different organ model will be combined to make a whole body-model.

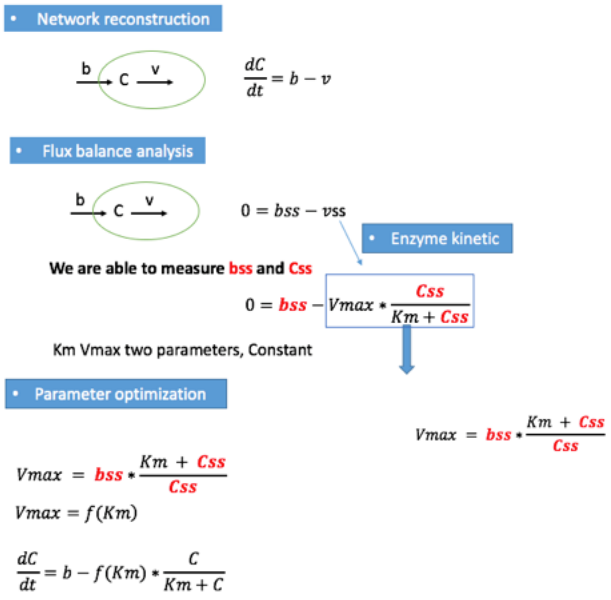

Figure 13.1 1 Modeling method summary

13.2 Step 1: Reconstruct the metabolite pathway

The metabolic patterns of the brain, muscle, adipose tissue, and liver are very different. This is fundamentally caused by different organs having different metabolites as well as different pathways.

Metabolites and enzymes involved in a reaction has been found in databases such as KEGG and Reactome (Rolfsson, Ottar 2011).

General human metabolic pathway has been developed by using a lot of mathematical approaches to reconstruct the complete metabolic states in humans. The most widely referred and cited one is Recon 1 (2002, Palsson et al).

Step for defining a human specific organ model:

1 draft reconstruction (bottom-up). There are now many different reconstructions published. From the very complicated and comprehensive generic information about metabolic function human model, special reaction is manually identified.

2 Manual curations for target organism. Examination of the 'standard' reaction and tailoring it into specific organs. Then, curation process for different organs is conducted. Obtaining the details of co-factor and physiological data that are available for the enzyme.

3 Some steps are needed to be lumped and this model is simplified because a good compromise between biological complexity and the simplicity needed for understanding and practical use should be found. For this model, all the rate determining steps and all the important choke points are kept. Quasi-Steady-State and Quasi Equilibrium Approximations (fast equilibrium will disappear) are used to simplify this model.

How these two approximations applied to this simple system are shown in figure 14.2.1 (Sa stands for ATP)

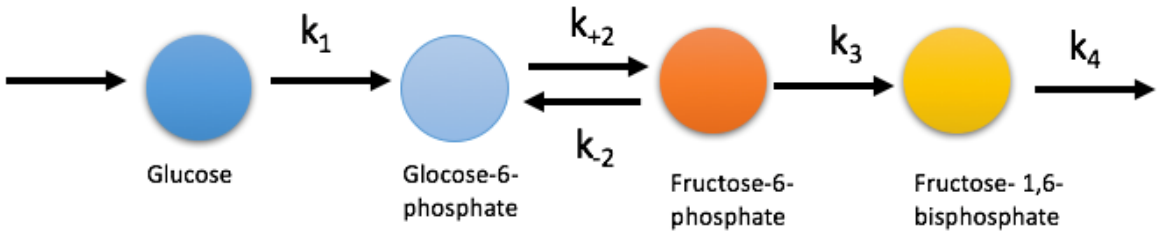

Figure 13.2 1 Reaction system

The differential equation can be written as:

$$\frac{ds1}{dt} = vo - k1 * sA * s1$$

$$\frac{ds2}{dt} = k1 * sA * s1 - k+2 * s2 + k-2 * s3$$

$$\frac{ds3}{dt} = k+2 * s2 - k-2 * s3 - k3 * sA * s3$$

$$\frac{ds4}{dt} = k3 * sA * s3 - k4 * s4$$

equation 13.2.1

That all reactions happen on a similar time scale is assumed, meaning that all the reaction rate constants (k) are on the same orders of magnitude.

Set k+2= k-2 = 2 and all other rate constants and the ATP concentration (sA) to values of 1 (arbitrary units). For time 0-5, system input flux should be vo = 2; then, the flux drops to vo =1. The simulation result is shown in figure 13.2.2.

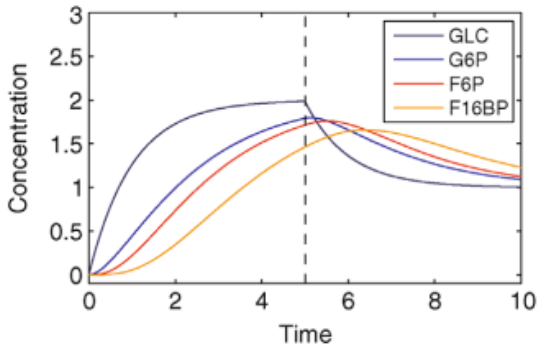

Figure 13.2 2 Results from the original model all k=1

Quasi-steady-state approximation: If the k1 increased to 5, glucose will be rapidly converted to g6p. Thus, it will adapt to the input flux change almost instantaneously. The glucose concentration at each time point is replaced by the steady-state value s1= v0/(k1*sA), substituting the differential equation of s1. Then all s1 concentrations appear in the following differential equations in equation 13.2.1. Here's the simulation result with (dash like) and without (solid) the assumption.

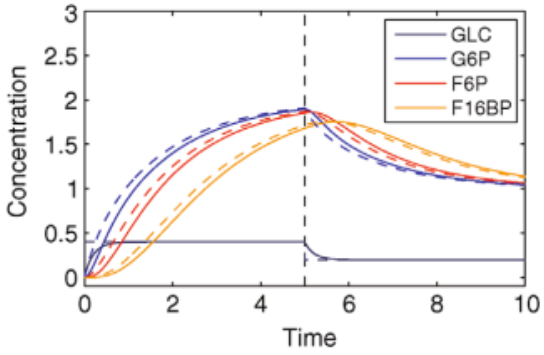

Figure 13.2 3 Results from the model with fast glucose turnover rate

k1=5 (solid lines) and the quasi-steady-state approximation (broken lines)

Quasi-equilibrium approximation: A rapid and reversible conversion between the G6P and F6P is considered. Keeping their ratio Keq= k+2/k-2. In the simulation, the ratio of F6P to G6P levels rapidly approaches the equilibrium constant [F6P]/[G6P] = s3/s2= Keq. Thus,

$$\frac{ds2+3}{dt} = \frac{d(s2+s3)}{dt} = k1 * sA * s1 - k3 * sA * s3$$

equation 13.2.2

Given s2 +3 and Keq, now s3 = s2+3 * Keq/(1+ Keq) is substituted in

$$\frac{ds4}{dt} = k3 * sA * s3 - k4 * s4$$

equation 13.2.3

and a simplified differential equation system in which the fast reaction has disappeared is obtained. The two differential equations for s2 or s3 are replaced by a single differential equation. Here is the simulation result with (dash line) and without (solid) the assumption.

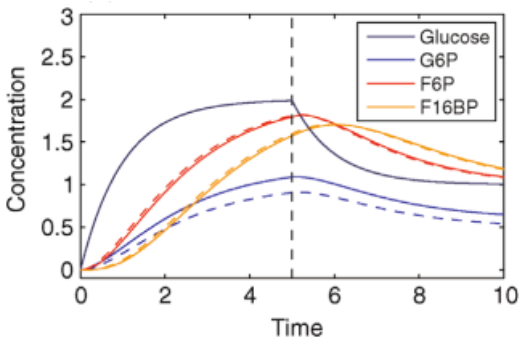

Figure 13.2 4 Results from the model with fast reversible conversion

G6P F6P (solid lines), parameters k+2 = 10; k-2 = 5, and the quasi-equilibrium approximation (dash lines)

13.3 Step 2: Flux Balance Analysis

For one very simple system.

$$\xrightarrow{b_1} x_1 \underset{v_{-1}}{\overset{v_1}{\rightleftharpoons}} x_2 \xrightarrow{b_2}$$

Figure 13.3 1 Reaction system

$$\frac{dx1}{dt} = b_1 - v_1 + v_{-1}$$
$$\frac{dx2}{dt} = v_1 - v_{-1} - b_2$$

equation 13.3.1

When this system reaches steady state, the concentration of x1 and x2 does not change any more. Thus, there are 2 steady state equations with 4 unknowns. Determining two of the unknowns will make it possible to solve the other two unknowns.

$$0 = b_1 - v_1 + v_{-1}$$
$$0 = v_1 - v_{-1} - b_2$$

equation 13.3.2

Due to the simplicity of the system, one can easily see that $b1 = v1 - v - 1 = b2$. This suggests us if one of the b1, b2 or v1+v-1 is known, one can get the rest two. Then by knowing one of the v1 or v-1, one can know the other.

By determining one of b1, b2 or v1+v-1, and one of v1 or v-1 the whole system can be solved.

The equations above are linear algebra. A stoichiometric matrix S can be formed based on these chemical equations. For differential equation for the same system equation 13.3.1 can be reformed as:

$$\frac{dx}{dt} = \boldsymbol{S} * v(x) = \begin{pmatrix} 1 & -1 & 1 & 0 \\ 0 & 1 & -1 & -1 \end{pmatrix} * \begin{pmatrix} b1 \\ v1 \\ v-1 \\ b2 \end{pmatrix} = \begin{pmatrix} b1 - v1 + v - 1 \\ v1 - v - 1 - b2 \end{pmatrix}$$

equation 13.3.3

The S is called stoichiometric matrix.

The quantities nij are the stoichiometric coefficients of the ith metabolite in the jth reaction.

$$\begin{matrix} & \text{b1} & \text{v1} & \text{v-1} & \text{b2} \\ \text{x1} \\ \text{x2} \end{matrix} \quad \mathbf{S} = \begin{pmatrix} 1 & -1 & 1 & 0 \\ 0 & 1 & -1 & -1 \end{pmatrix}$$

Figure 13.3 2 S matrix

For this specific system (figure13.3.1), S is a 2-by-4 matrix (m*n matrix), meaning that it has 2 metabolites and 4 unknown fluxes. S with a rank r = 2, standing for the 2 linear independent equations can be written down because of 2 (m =2) metabolite chemical reactions (equation 13.3.1). There are 4 (n =4) columns in S indicating 4 unknown fluxes. In steady state, $\frac{dx}{dt} = S * vss = 0$. vss resides in the null space of S. Dimension of Dim(Null(S)) = n - r = 2 revealing that there are two free parameters for the steady state solutions of equation 14.3.1. The result of Null space of S:

$$Null(S) = \begin{pmatrix} 1 & 0 \\ 1 & 1 \\ 0 & 1 \\ 1 & 0 \end{pmatrix}$$

equation 13.3.4

All steady state flux states of the system are a non-negative combination of these two vectors:

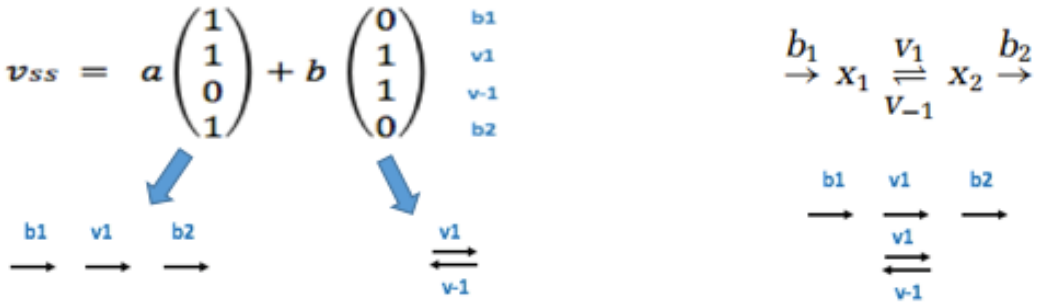

Figure 13.3 3 Null space

It is not hard to find that this is another form of equation 13.3.2. One can substitute b1 = a, v1= a + b, v-1= b, b2 =a, in equation 13.3.2 and get that

$$b1 - v1 + v - 1 = a - (a + b) + b$$

$$= 0$$

$$v1 - v - 1 - b2 = (a + b) - a - b$$

$$= 0$$

Proving that matrix offering the same solution as equation 13.3.2.

Matrix is another form of setting up equations fundamentally, which helps us keep track of meaningful information of the system. The summary of equation form and matrix form is in the picture.

	Equation Form	Matrix Form
Differential equation	$\frac{dx1}{dt} = b_1 - v_1 + v_{-1}$ $\frac{dx2}{dt} = v_1 - v_{-1} - b_2$	$\frac{dx}{dt} = \boldsymbol{S} * v(x) = \begin{pmatrix} 1 & -1 & 1 & 0 \\ 0 & 1 & -1 & -1 \end{pmatrix} * \begin{pmatrix} b1 \\ v1 \\ v-1 \\ b2 \end{pmatrix}$
Steady state equation	$0 = b_1 - v_1 + v_{-1}$ $0 = v_1 - v_{-1} - b_2$	$v_{ss} = a\begin{pmatrix} 1 \\ 1 \\ 0 \\ 1 \end{pmatrix} + b\begin{pmatrix} 0 \\ 1 \\ 1 \\ 0 \end{pmatrix}$ b1 v1 v-1 b2
Steady state solution	Four unknowns and two equations, Thus there would be two free unknowns without introducing objective functions. After rearranging equations we find that, $b1 = b2$, knowing one of them will get the other. Knowing one more from v1 or v-1 will Solve the system.	$v_{ss} = a\begin{pmatrix} 1 \\ 1 \\ 0 \\ 1 \end{pmatrix} + b\begin{pmatrix} 0 \\ 1 \\ 1 \\ 0 \end{pmatrix}$ b1 v1 v-1 b2 We can easily see that b1 = b2 and knowing one of them will get the other. Knowing another one of v1 or v-1 can determine the system.

Figure 13.3 4 Comparison between equation form and matrix form

The strength is not obvious in this simple system. However, one can explore more complex system and see how stoichiometric matrix help us understand the relation in glycolysis pathway (Bernhard O. Palsson, system biology).

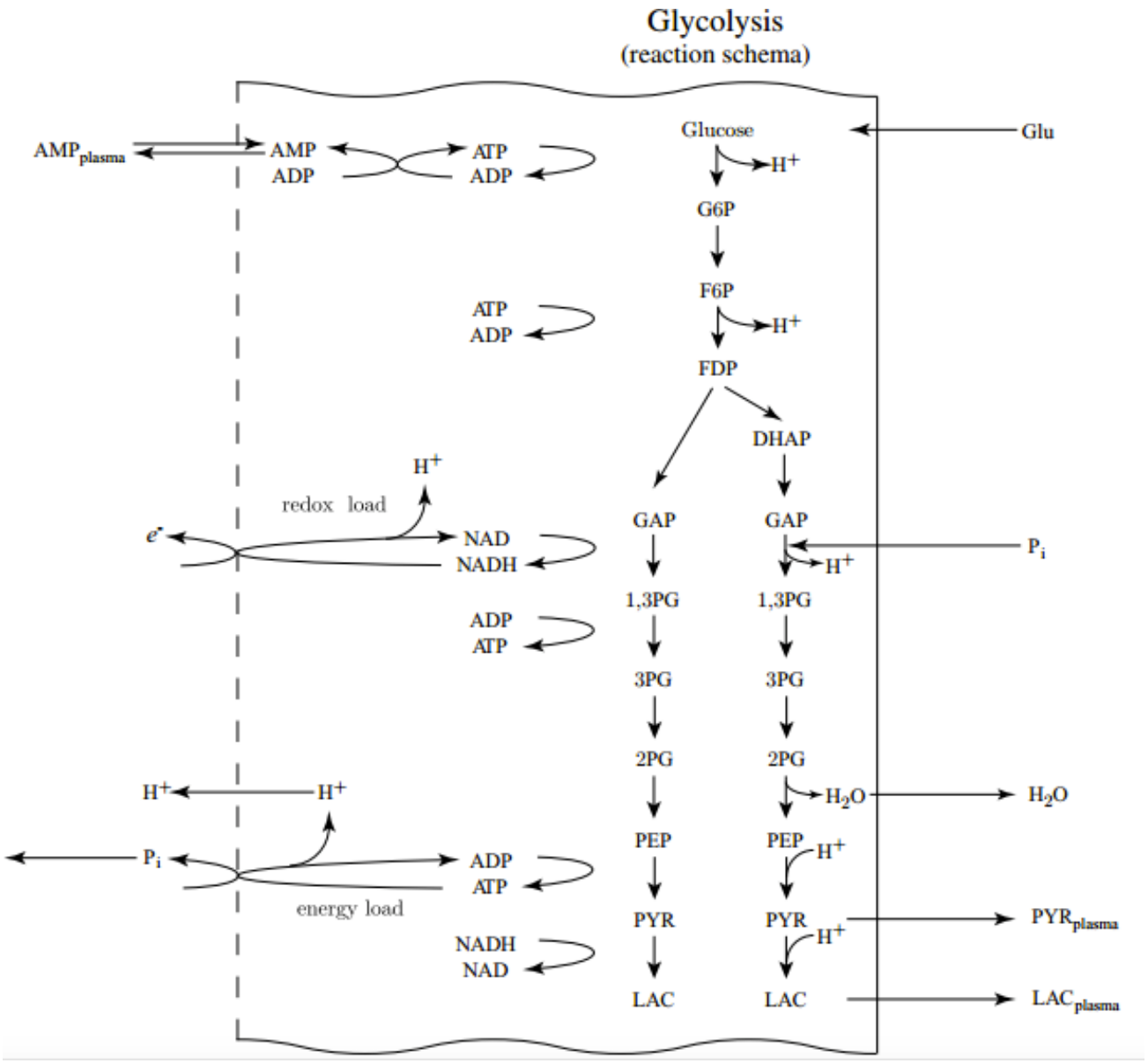

Figure 13.3 5 Glycolysis

Here's the Mathematica simulation for solving steady state flux using matrix form

(1) Define stoichiometric matrix. There are m=20 compounds and n=21 reactions. S is a 20*21 matrix. (In real system, there are always more reactions than equations.)

```
variables = {glu, g6p, f6p, fbp, dhap, gap, pg13, pg3, pg2, pep, pyr, lac, nad,
    nadh, amp, adp, atp, phos, h, h2o};
varnames = {"glu", "g6p", "f6p", "fbp", "dhap", "gap", "pg13", "pg3", "pg2", "pep",
    "pyr", "lac", "nad", "nadh", "amp", "adp", "atp", "phos", "h", "h2o"};

fluxes = {vhk, vpgi, vpfk, vtpi, vald, vgapdh, vpgk, vpglm, veno, vpk, vldh, vamp,
    vapk, vpyr, vlac, vatp, vnadh, vgluin, vampin, vh, vh2o};

fluxnames = {"vhk", "vpgi", "vpfk", "vtpi", "vald", "vgapdh", "vpgk", "vpglm",
    "veno", "vpk", "vldh", "vamp", "vapk", "vpyr", "vlac", "vatp", "vnadh",
    "vgluin", "vampin", "vh", "vh2o"};

  MatrixForm[
    stoich = {{-1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0},
              {1, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
              {0, 1, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
              {0, 0, 1, 0, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
              {0, 0, 0, -1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
              {0, 0, 0, 1, 1, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
              {0, 0, 0, 0, 0, 1, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
              {0, 0, 0, 0, 0, 0, 1, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
              {0, 0, 0, 0, 0, 0, 0, 1, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
              {0, 0, 0, 0, 0, 0, 0, 0, 1, -1, 0, 0, -1, 0, 0, 0, 0, 0, 0, 0, 0},
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              {0, 0, 0, 0, 0, -1, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0},
              {0, 0, 0, 0, 0, 1, 0, 0, 0, 0, -1, 0, 0, 0, 0, 0, -1, 0, 0, 0, 0},
              {0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, -1, 1, 0, 0, 0, 0, 0, 1, 0, 0},
              {1, 0, 1, 0, 0, 0, -1, 0, 0, -1, 0, 0, -2, 0, 0, 1, 0, 0, 0, 0, 0},
              {-1, 0, -1, 0, 0, 0, 1, 0, 0, 1, 0, 0, 1, 0, 0, -1, 0, 0, 0, 0, 0},
              {0, 0, 0, 0, 0, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0},
              {1, 0, 1, 0, 0, 1, 0, 0, 0, -1, -1, 0, 0, 0, 0, 1, 1, 0, 0, -1, 0},
              {0, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, -1, 0, 0, 0, 0, -1}}
  ];

  MatrixForm[stoich, TableHeadings → {varnames, fluxnames}]

  dim = Dimensions[stoich, 2];
  rank = MatrixRank[stoich];
```

(2) Get the null space of ***S,*** the rank of S is r=18, so one can expect that the Null space is 3,

Dim[Null(S)]] = n - r=21-18 = 3.

```
null = NullSpace[stoich];
pathwaynames = {"p1", "p2", "p3"};
MatrixForm[null, TableHeadings → {pathwaynames, fluxnames}]
```

(3) (Optional) Rearranging Null space so that the matrix looks neat.

```
MatrixForm[-{{1, 0, 1}, {1, 0, 0}, {0, 1, 0}}.null, TableHeadings → {pathwaynames, fluxnames}]
```

result from step1

	vhk	vpgi	vpfk	vtpi	vald	vgapdh	vpgk	vpglm	veno	vpk	vldh	vamp	vapk	vpyr	vlac	vatp	vnadh	vgluin	vampin	vh	vh2o
glu	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
g6p	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
f6p	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fbp	0	0	1	0	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
dhap	0	0	0	-1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
gap	0	0	0	1	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pg13	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pg3	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0
pg2	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0
pep	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0
pyr	0	0	0	0	0	0	0	0	0	1	-1	0	0	-1	0	0	0	0	0	0	0
lac	0	0	0	0	0	0	0	0	0	0	1	0	0	0	-1	0	0	0	0	0	0
nad	0	0	0	0	0	-1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
nadh	0	0	0	0	0	1	0	0	0	0	-1	0	0	0	0	0	-1	0	0	0	0
amp	0	0	0	0	0	0	0	0	0	0	0	-1	1	0	0	0	0	0	1	0	0
adp	1	0	1	0	0	0	-1	0	0	-1	0	0	-2	0	0	1	0	0	0	0	0
atp	-1	0	-1	0	0	0	1	0	0	1	0	0	1	0	0	-1	0	0	0	0	0
phos	0	0	0	0	0	-1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
h	1	0	1	0	0	1	0	0	0	-1	-1	0	0	0	0	1	1	0	0	-1	0
h2o	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	-1	0	0	0	0	-1

result from step2

	vhk	vpgi	vpfk	vtpi	vald	vgapdh	vpgk	vpglm	veno	vpk	vldh	vamp	vapk	vpyr	vlac	vatp	vnadh	vgluin	vampin	vh	vh2o
p1	0	0	0	0	0	0	0	0	0	0	1	0	0	-1	1	0	-1	0	0	-2	0
p2	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	0	0	0	-1	0	0
p3	-1	-1	-1	-1	-1	-2	-2	-2	-2	-2	-3	0	0	1	-3	-2	1	-1	0	0	0

result from step3

	vhk	vpgi	vpfk	vtpi	vald	vgapdh	vpgk	vpglm	veno	vpk	vldh	vamp	vapk	vpyr	vlac	vatp	vnadh	vgluin	vampin	vh	vh2o
p1	1	1	1	1	1	2	2	2	2	2	2	0	0	0	2	2	0	1	0	2	0
p2	0	0	0	0	0	0	0	0	0	0	-1	0	0	1	-1	0	1	0	0	2	0
p3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0

Take human red blood cell as an example. The uptake rate of the red blood cell of glucose is about 1.12 mM/h (That will be 1.12*p1). The input of AMP is measured to be 0.014 mM/h. (That will be 0.014*p3). The steady state load on NADH is 0.224 mM/h.(So 0.224*p2).

$$vss= 1.12*p3+0.224*p2+ 0.014*p3$$

In this model – for a bunch of complex pathways for each organ, matrix is going to give us useful information to help to solve the steady state.

Above all the flux balance, the whole body has another layer of balance. The balance between different organs. For example, the total amount of glucose each organ uses to keep it functional should equate the total amount of glucose that liver produces.

This should be true for all the macronutrient which can go into and out of the cell. (Including glucose, pyruvate, lactate, amino acid, glycerol, triglyceride).

Gathered are data for human overnight (typically 10 hours after dinner) macronutrient uptake and release rate. Adjust them within the physiology range to make sure one can get a balance between the organs that are about to be simulated.

v	GLC	PYR	LAC	ALA	FFA	GLR	TG
Brain	0.38	0	0	0	0	0	0
Heart	0.04	0	0.04	0	0.035	0	0
Liver	-0.731	0	0.27	0.32	0.21	0.14	-0.029
GI_track	0.076	0	0	0	-0.12	-0.04	0.006
Muscle	0.165	0.005	-0.112	-0.04	0.046	-0.003	0.003
Adipose	0.038	0	-0.056	0	-0.211	-0.097	0.02
Others	0.032	-0.005	-0.142	-0.28	0.04	0	0
Sum	0	0	0	0	0	0	0

Table 13.3 1 Macronutrient Uptake and release rates (mmol min-1) in each tissue/organ system

This will make sure that one can have a steady state for the whole body when later on the models are connected.

13.4 Step 3: Express calculated flux with respect to metabolite concentration

Enzymatic activity's IRFE (Intracellular Reaction Flux Expression) involves several physiologically-based mathematical terms: In general, they are concentration of substrate (glucose etc.), phosphorylation state (ATP, ADP, AMP, ATP/ADP etc.), redox state (NAD, NADH, NAD/NADH etc.), product or third party allosteric stimulation and/or inhibition (AcCoA etc.). The IRFE assumes random sequential reaction.

Hormonal actions (insulin/glucagon) can be expressed to directly affect enzymatic Vmax for each IRFE: Physiologically, insulin/glucagon do not go inside the cells but rather bind to 'insulin receptor' and 'glucagon receptor' which can stimulate/inhibit downstream signal processes. These signals (ie. PCK, FOXO etc., which were not considered at all in IRFE) affect IRFE; however, this problem can be temporarily circumvented if one assumes and expresses IRFE being stimulated/inhibited directly by hormonal actions.

Two major parameters of interest are Km and Vmax for each IRFE.

Km may be easier to get values than Vmax. They can be obtained from literature (most preferable and most widely used in research), optimized with multiple data points with manual adjustment (second most widely used), or assumed to be steady-state concentration of corresponding substrate/product.

Hormones (insulin/glucagon/adrenaline etc.) and oxygen level (exercise etc.) can affect IRFE of glycolysis, TCA and other reactions via signaling. Blood flow rate can be also affected.

Enzyme kinetics is the study of chemical reactions that are catalyzed by enzyme. As enzyme-catalysed reactions are saturable, their rate of catalysis does not show a linear response to

increasing substrate (do not show the same pattern like first order reaction). Here several enzyme kinetics and their rate equations in term of substrate concentration are introduced.

Competitive inhibition.

Competitive inhibitors compete for the substrate-binding site of the enzyme with the substrate as illustrated in figure 13.4.1

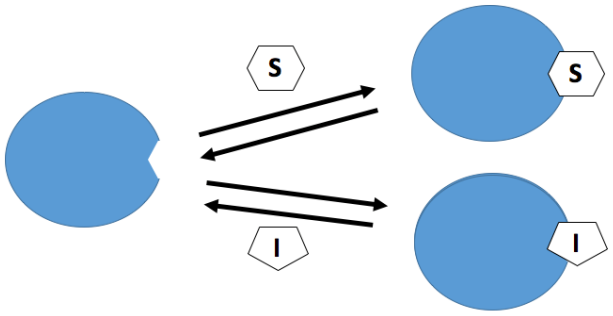


Figure 13.4 1 Competitive inhibition

There are

$$E+S \rightleftharpoons ES \rightleftharpoons E+P$$

$$E+I \rightleftharpoons EI$$

Thus the Michaelis-Menten equation for this becomes:

$$v = vmax * \frac{[S]}{km * \left(1 + \frac{[I]}{ki}\right) + [S]}$$

Uncompetitive inhibition

Some inhibitors bind only to the ES complex without binding to the free enzyme. This interaction scheme is illustrated in figure 13.4.2

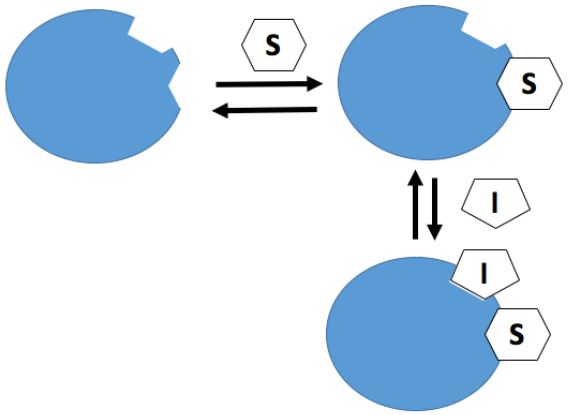


Figure 13.4 2 Uncompetitive inhibition

There are

$$E+S \rightleftharpoons ES \rightleftharpoons E+P$$

$$ES+I \rightleftharpoons EIS$$

Thus the Michaelis-Menten equation for this becomes:

$$v = vmax * \frac{\frac{[S]}{1 + \frac{[I]}{ki}}}{\frac{km}{\left(1 + \frac{[I]}{ki}\right)}}$$

Noncompetitive inhibition

In noncompetitive inhibition, the inhibitor binds to the enzyme at a location other than the active site in such a way that the inhibitor and substrate can simultaneously be attached to the enzyme.

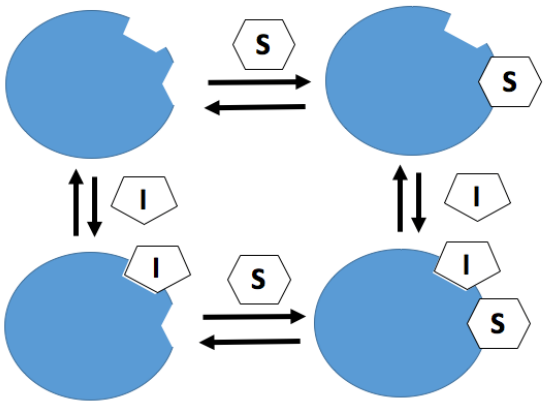


Figure 13.4 3 noncompetitive inhibition

so there are

E+S⇋ES⇋E+P

E+I⇋EI

ES+I⇋EIS

EI+S⇌EIS

Thus the Michaelis-Menten equation for this becomes:

$$v = \frac{vmax * [S]}{km\left(1 + \frac{[I]}{ki}\right) + [S](1 + \frac{[I]}{ki})}$$

Ping pong (bi-bi) mechanism

Ping-pong mechanism, also called a double-displacement reaction, or bi-bi reaction is characterized by the change of the enzyme into an intermediate form when the first substrate to product reaction occurs. It is important to note the term intermediate indicating that this form is only temporary. At the end of the reaction the enzyme MUST be found in its original form. An enzyme is defined by the fact that it is involved in the reaction and is not consumed. Another key characteristic of the ping-pong mechanism is that one product is formed and released before the second substrate binds. The figure below explains the Ping Pong mechanism through an enzymatic reaction.

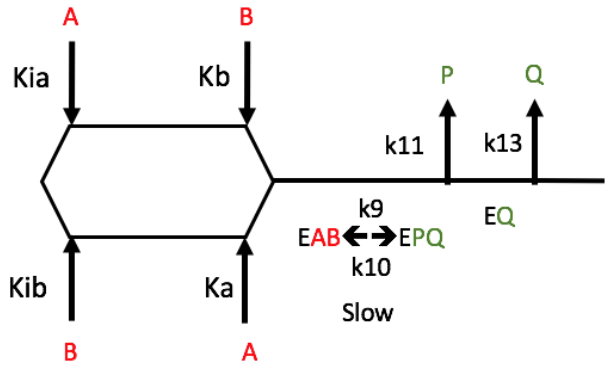


Figure 13.4 4 Bi-bi mechanism

The Michaelis-Menten equation for this can be written as:

$$v = \frac{vmax * [A] * [B]}{kia * kb + kb * [A] + ka * [B] + [A] * [B]}$$

The four enzyme kinetics above are the most frequently used ones in this research's system. Other enzyme kinetic equations that include but limit to activation, reversible catalysis also are part of the equations.

13.5 Step 5: parameter optimization

For this research's differential system, one can use time vs. concentration to fit the parameters.

However, there are always more than ten parameters. Using one group of concentration vs time data may not be valid enough. Therefore, the research came up with using metabolite concentration range, which is the easiest type of parameter to obtain, to optimize the parameters.

The number of metabolite concentration range should be similar or larger than number of unknown parameters.

$$\frac{dC}{dt} = b - v$$

$$= b - Vmax * \frac{C}{Km + C}$$

steady state bss, Css

$$0 = bss - Vmax * \frac{Css}{Km + Css}$$

Thus,

$$Vmax = bss * \frac{Km + Css}{Css}$$

$$\frac{dC}{dt} = b - (bss * \frac{Km + Css}{Css}) * \frac{C}{Km + C}$$

Let's assume bss = 1, Css = 1

$$\frac{dC}{dt} = b - (1 + Km) * \frac{C}{Km + C}$$

plot $v = (1 + \mathrm{Km}) * \frac{\mathrm{C}}{\mathrm{Km+C}}$ with different Km value. Because of the steady state, Vmax depends on Km, all those different lines intersect at (1, 1)

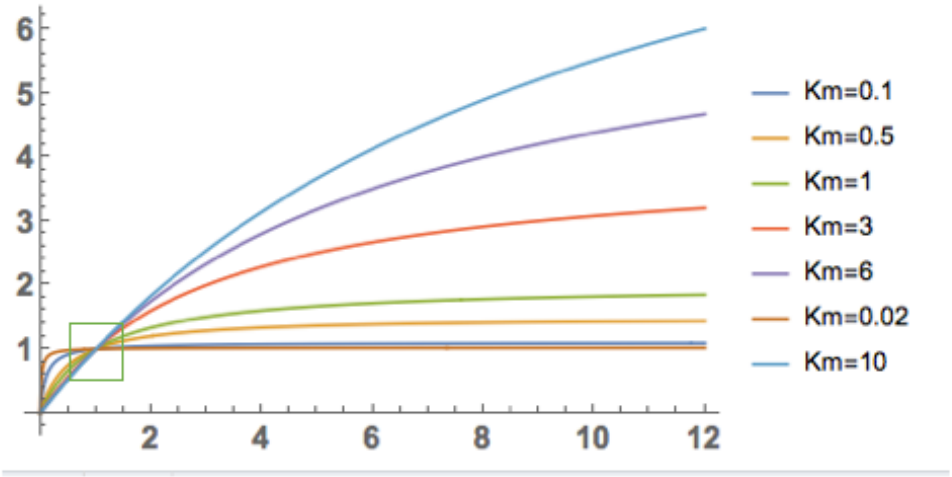


Figure 13.5 1 Different Vmax and Km combinations

Enlarging the part within the green box, and if one perturbs the steady state, a new steady state is obtained. New steady state flux range is 0.995 to 1.005.

And if the concentration range is 0.98 to 1.03 as shown in the figure below, those Km falling out of the black line (new steady state range) are not valid Km.

Intracellular Metabolite Concentration In Each Tissue						
Substrate\Tissues	Brain	Heart	Muscle	GI	Liver	Adipose
GLC	1.12	1	0.48	1	8	2.54
PYR	0.15	0.2	0.048	0.2	0.37	0.37
LAC	1.45	3.88	1.44	3.88	0.82	0.82
ALA	0	0	1.3	0	0.23	0

GLR	0	0.015	0.064	0.015	0.07	0.22
FFA	0	0.021	0.53	0.021	0.57	0.57
TG	0	3.12	14.8	450	2.93	990
O2	0.027	0.96	0.49	0.49	0.027	0.027
CO2	15.43	20	15.43	15.43	15.43	15.43
G6P	0.16	0.17	0.24	0.17	0.2	0.2
GLY	2	33	95	33	417	0
GAP	0.15	0.01	0.08	0.01	0.11	0.11
GRP	0	0.29	0.15	0.29	0.24	0.24
ACoA	0.068	0.0012	0.0022	0.0012	0.035	0.035
CoA	0.06	0.012	0.018	0.012	0.14	0.14
NAD	0.064	0.4	0.45	0.4	0.45	0.45
NADH	0.026	0.045	0.05	0.045	0.05	0.05
ATP	2.45	3.4	6.25	3.4	2.74	2.74
ADP	0.54	0.02	0.02	0.02	1.22	1.22
Pi	2.4	1.66	2.7	1.66	4.6	4.6
PCR	4.6	8.3	20.1	8.3	0	0
CR	5.6	3.5	10.45	3.5	0	0

Table 13.5 1 Substrate concentrations (mM) in each tissue

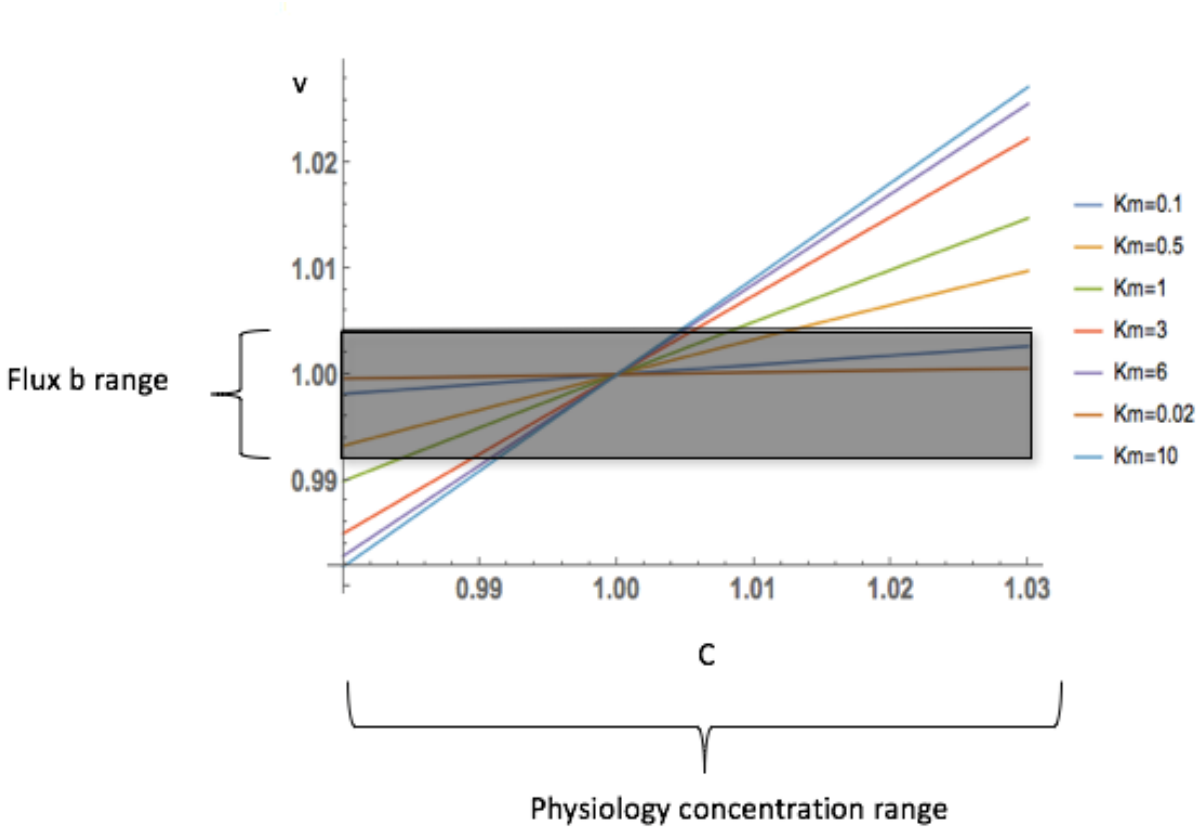
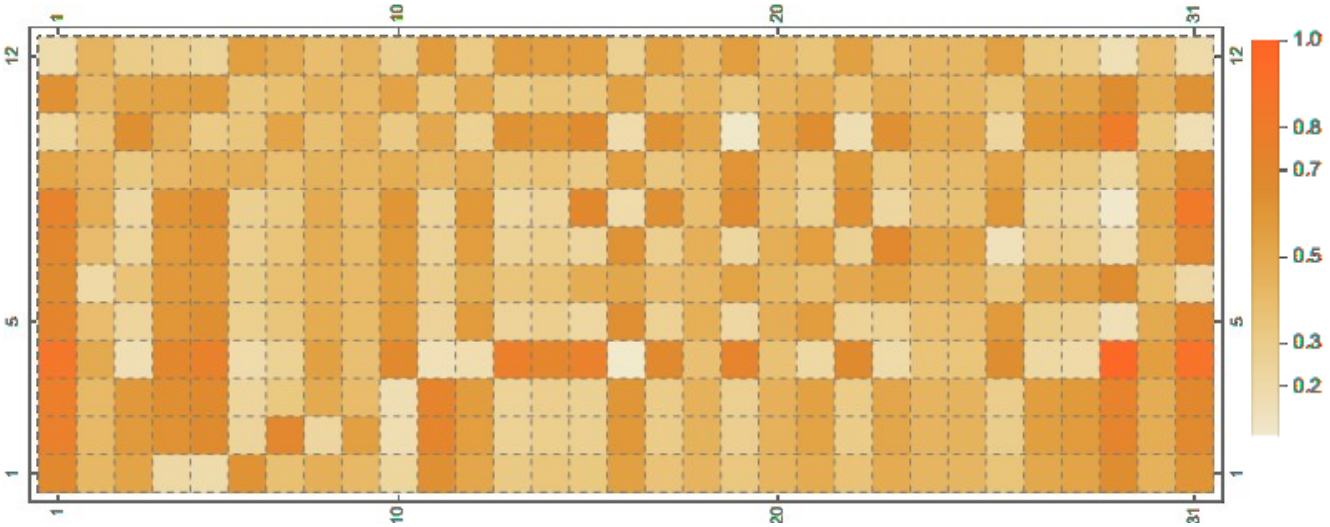


Figure 13.5 2 Optimization with range

13.6 Step 6: Performing sensitivity analysis on the parameters estimated



{Kglut2 → 20 000, Klaccb → 1000, Kpyrcb → 5000, Kgkrp → 15 000, Kgk → 7500, Kgkin → 10,
Keqgpi → 0.5157, Kg6p → 182, Kf6p → 71, Klump1 → 16, Klump1ps → 0.677998, Kpk → 44.5326,
Kpkcoa → 30, Kpkps → 5.61757, Kpdc → 220, Kpdccoa → 35, Kpdcrs → 4.42534, Keqldh → 17 606.1,
Kldh → 495, Kldhlac → 31 980, Kldhnad → 984, Kldhnadh → 27, Kpc → 220, Kpccoa → 0.63,
Kpcps → 0.0356755, Kpepck → 33, Ktca → 0.4, Ktcaoxa → 0.045, Ktcaadp → 410,
Koxrs → 0.0176719, Koxps → 82.5687, xglub → 4400, xpyrb → 68, xlactateb → 192}

Figure 13.6 1 Sensititivity analysis

Sensitivity analysis is measurement of body's exposure to a drug, nutrient or metabolite as a result of a parameter change, under different conditions (postprandial, fasting etc). The parameter change can be marginal or big. The degree of body's exposure can be defined as Area Under the Curve (AUC) which computes time integral between initial and end concentration or flux. Sensitivity analysis can be performed at a fixed time or be transient.

In this research's case, there are more than 100 parameters which have to be obtained through literature search, educated guess based on diffusion or convection, or computational optimization against clinical and experimental data. Out of these parameters, there may be some parameter

changes that change AUC more severely than others. The sensitive parameters can be further optimized to better fit the simulated plots against real physiological data while less sensitive parameters can be either lumped and optimized or disregarded for further optimization.

The chosen sensitive parameters then can be used for analysis to figure out which reaction is more responsible for the change of AUC in both healthy and T2D patients. In case of T2D patients, such information can be used to develop drug which target a reaction of a certain organs/tissues/cells. For example, if one figures out which reaction in gluconeogenesis is more responsible, one can further fine tune already existing drugs such as metformin or even further 'cure' T2D.

14. MODEL RESULTS

14.1 Pancreas α Cell

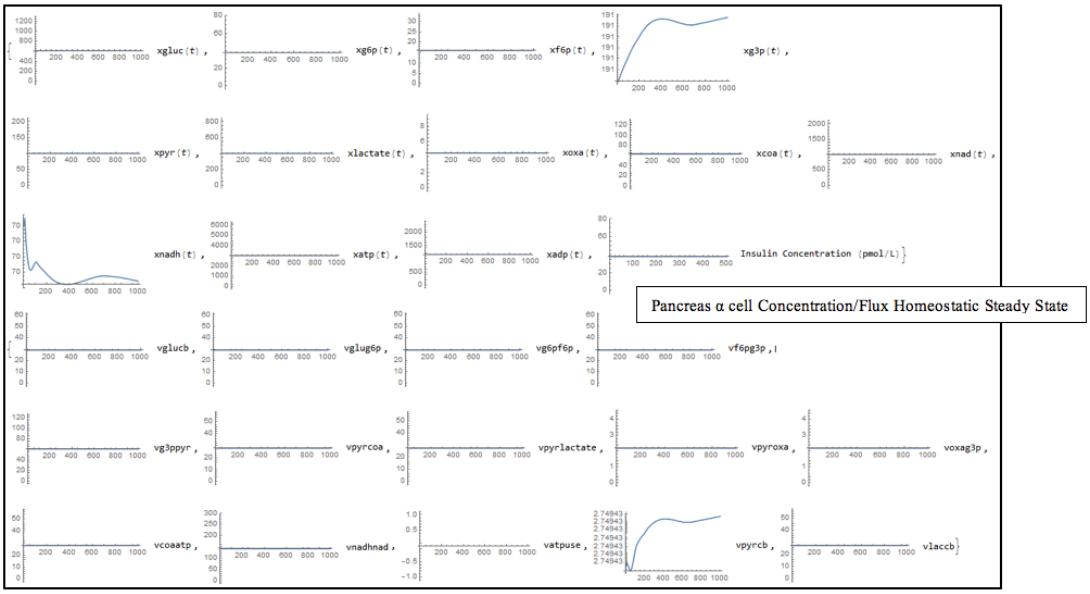


Figure 14.1 1 α cell steady state

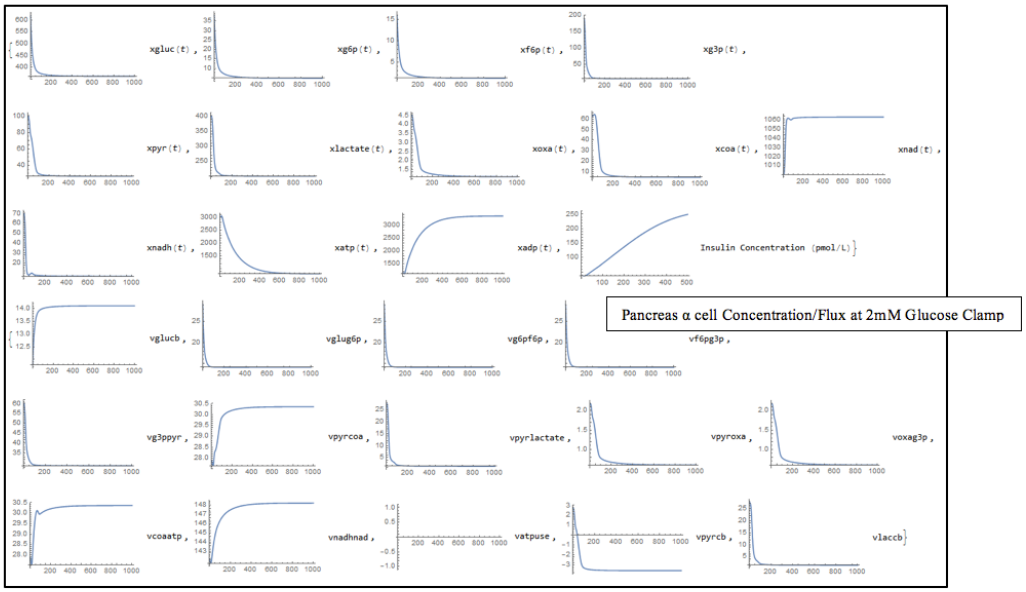


Figure 14.1 2 α cell at 2mM glucose clamp

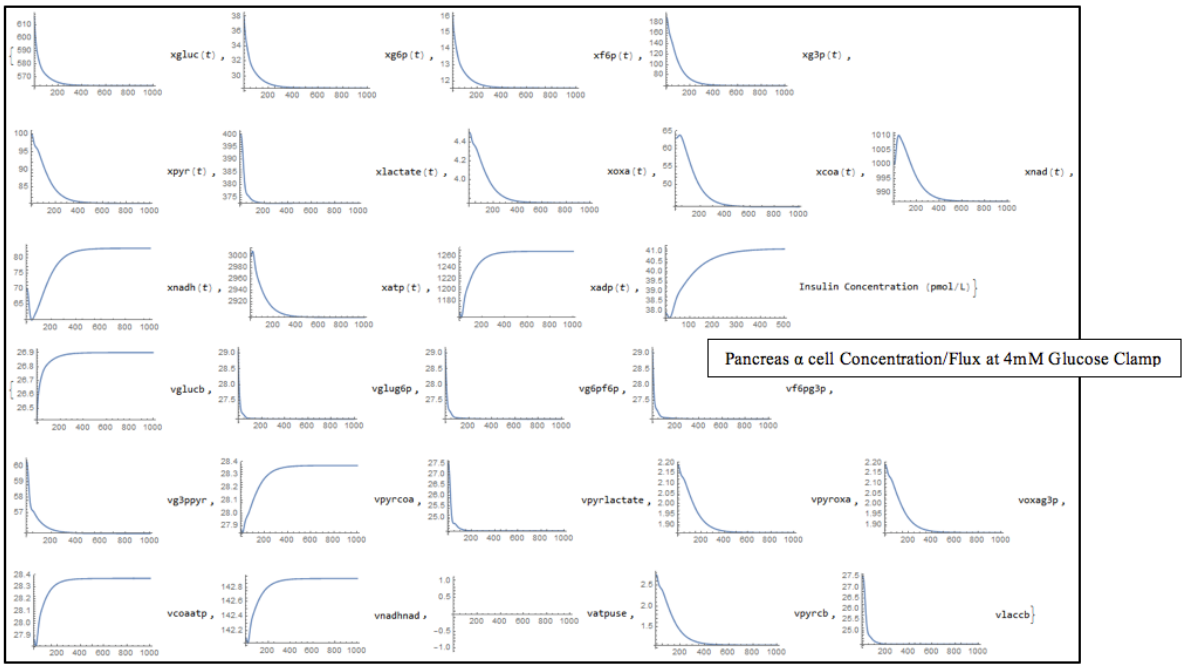


Figure 14.1 3 α cell 4mM glucose clamp

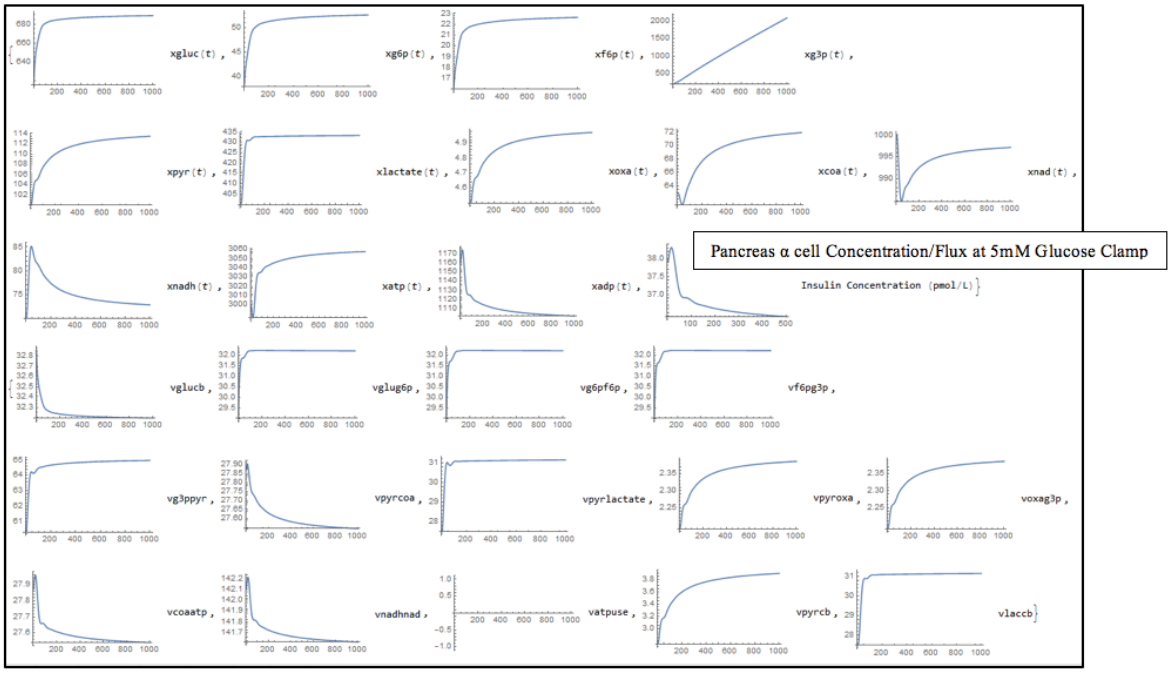


Figure 14.1 4 α cell 5mM glucose Clamp

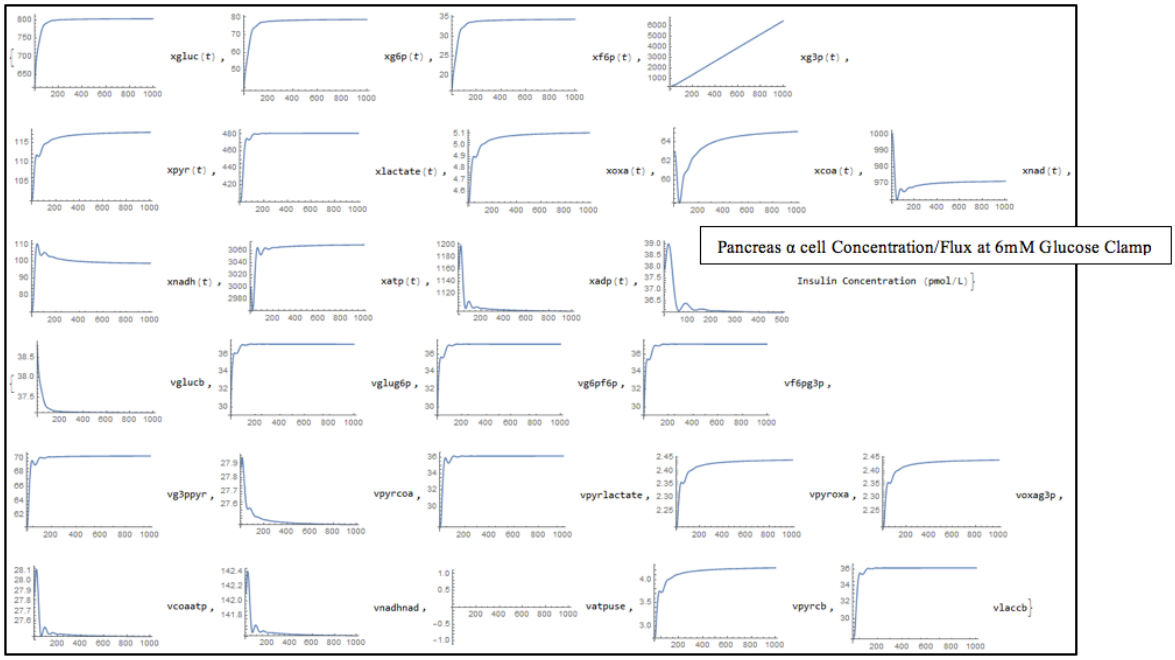


Figure 14.1 5 α cell 6mM glucose clamp

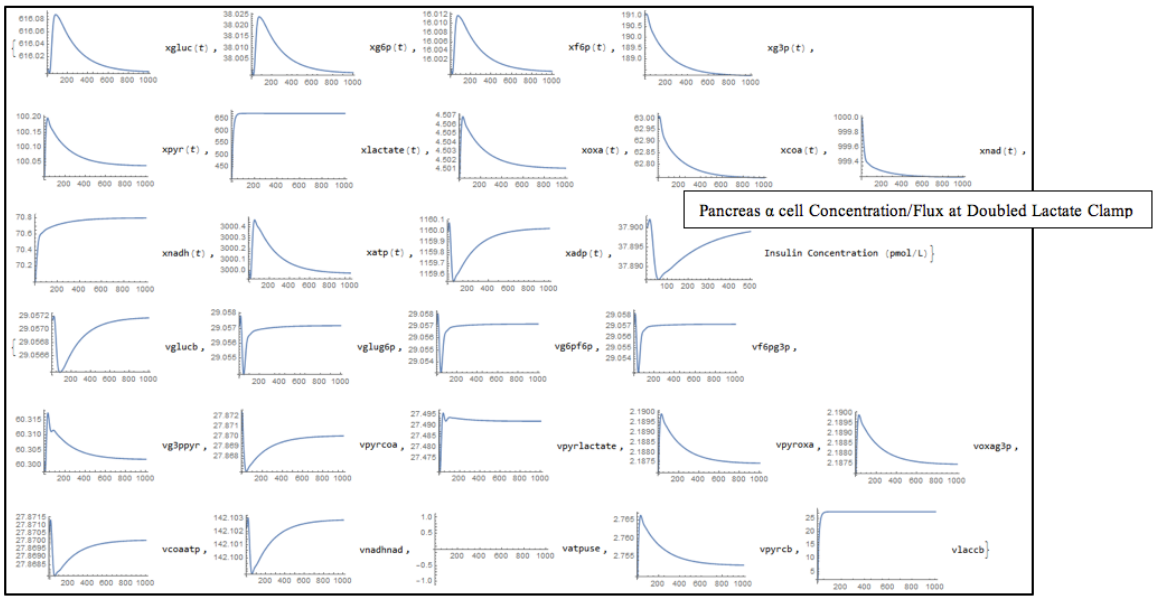


Figure 14.1 6 α cell double lactate clamp

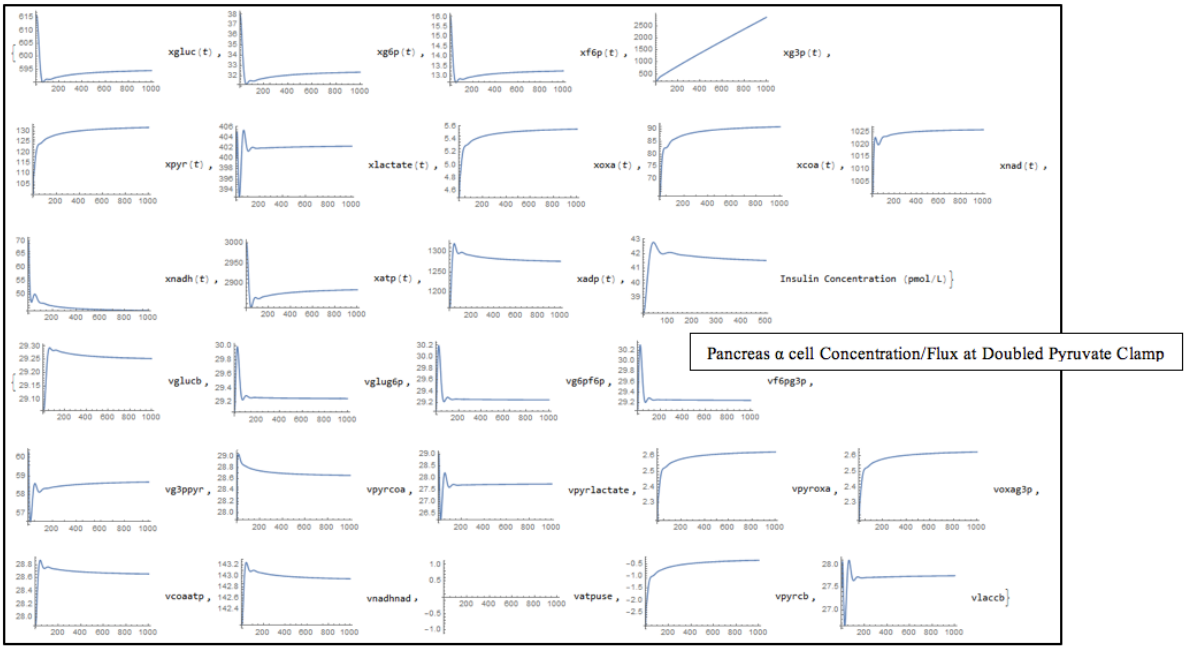


Figure 14.1 7 α cell double pyruvate clamp

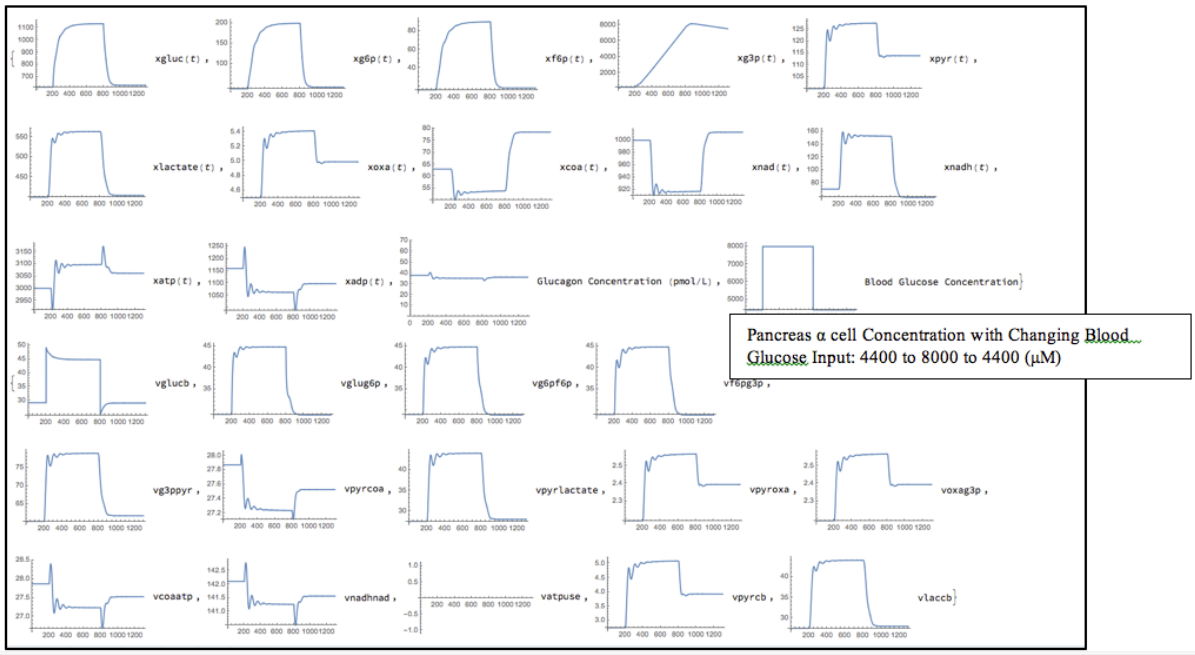


Figure 14.1 8 α cell with glucose input 4.4mM to 8mM to 4.4mM

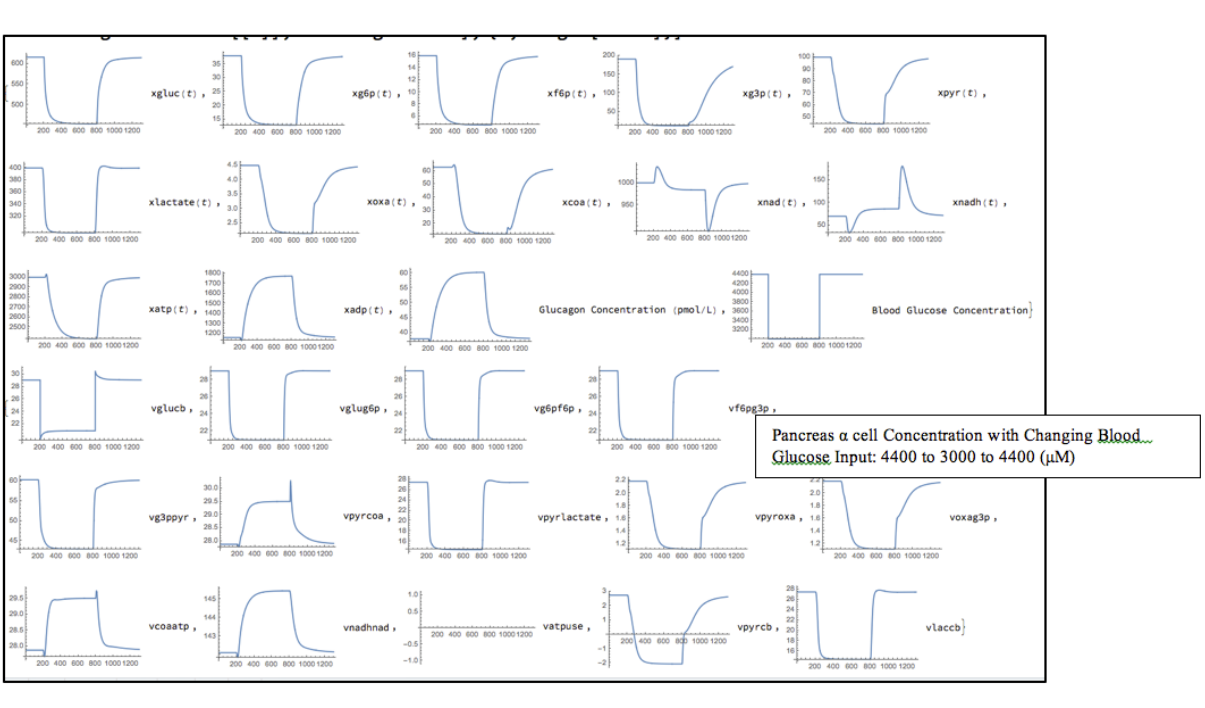


Figure 14.1 9 α cell with glucose input 4.4mM to 3mM to 4.4mM

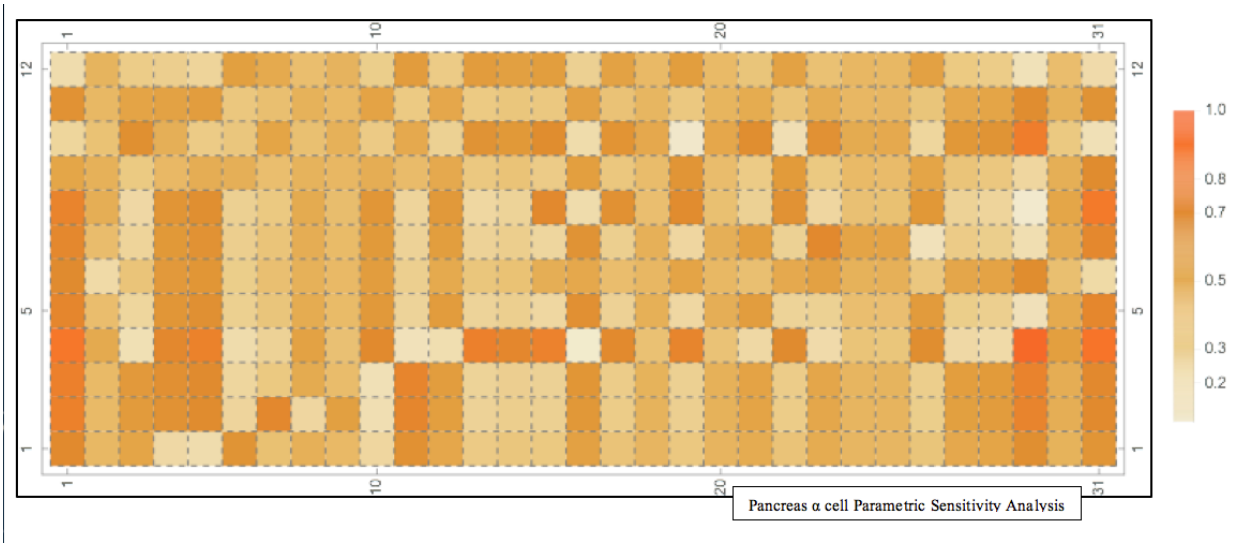


Figure 14.1 10 α cell sensitivity analysis

Figure 14.1.1 shows what happen to intracellular concentration of metabolites and fluxes during homeostasis. Every concentration and flux stays the same at its steady state value. This is an

expected behavior because the blood macronutrient concentration is kept constant by infusion. The most important profile is glucagon secretion rate. Under different conditions (hypoglycemia, hyperglycemia, doubled lactate/pyruvate concentrations and fluctuating blood glucose concentrations), the α cell's glucagon secretion rate will change accordingly. The decrease in ATP:ADP ratio will stimulate α cell to secrete more glucagon to increase blood glucose level. This is in contrast with β cell.

Figure 14.1.2 shows hypoglycemic condition and corresponding glucagon secretion rate. The glucagon is expected to increase a lot especially at 2mM glucose concentration (very low glucose level). The secretion profile shows steep increase in glucagon secretion within 500 seconds. This fits very well with physiological data.

Figure 14.1.3 shows slight hypoglycemic condition and corresponding glucagon secretion rate. The secretion still happens but not quite as fast. Both figure 14.1.2 and 14.1.3 show drastic contrast compared to figure 14.1.4 and 14.1.5. The glucagon secretion rate for the latter case (hyperglycemic) decreases to a level lower than the initial rate. This shows that glucagon secretion is hindered by detailed mechanism in a human body when blood glucose level is high.

Figure 14.1.6 and 14.1.7 are from doubled lactate and pyruvate clamp experiments in a human body. There are changes in glucagon secretion rate. Lactate concentration increase does not affect the secretion rate much while pyruvate concentration increase affects secretion rate by a few percent. This difference may be due to pyruvate's metabolic pathway which leads to lower ATP:ADP ratio compared to that of lactate's.

Figure 14.1.8 and 14.1.9 respectively shows fluctuating blood glucose concentration over time. The former shows intermittent glucose level increase while the latter shows the intermittent

glucose level decrease. These show the corresponding glucagon secretion rate fluctuation and demonstrates that the model works well without stiffness. The contrast between figure 14.1.8, where glucagon secretion rate is almost the same at homeostatic to hyperglycemic level, and figure 14.1.9, where glucagon secretion rate shoots up and boils down is remarkable.

Lastly, figure 14.1.10 shows the sensitivity analysis of α cell. 20% change in parameters (vertical) lead to changing exposure of metabolites (area under the curve, AUC) to a human body (horizontal). The redder the color is, the exposure becomes larger while the whiter it is, the exposure becomes minimal. The sensitivity values are normalized so that the maximum absolute value should be 1.

14.2 Pancreas β Cell

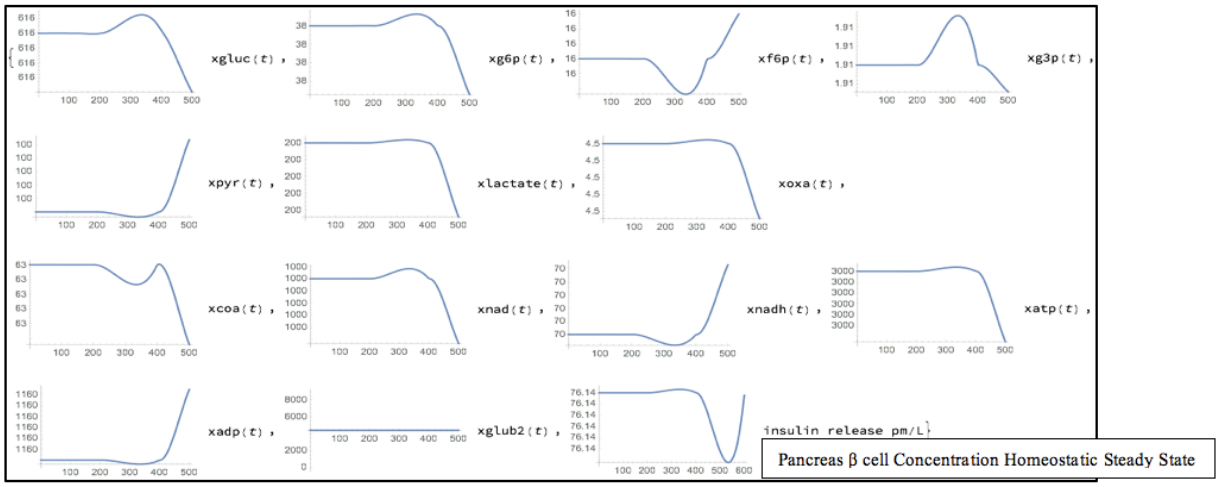


Figure 14.2 1 β cell steady state

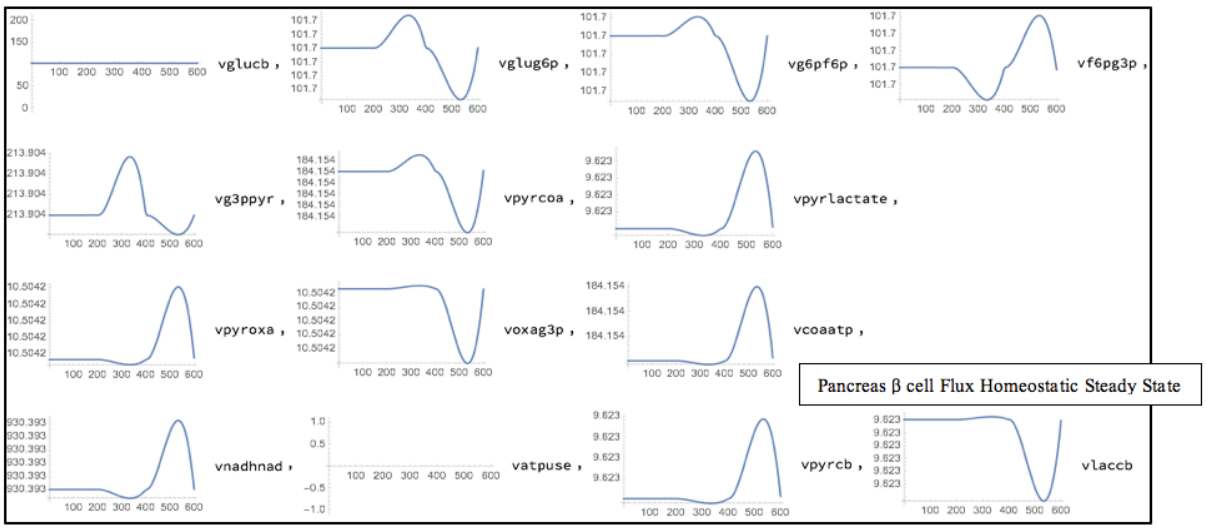


Figure 14.2 2 β cell steady state

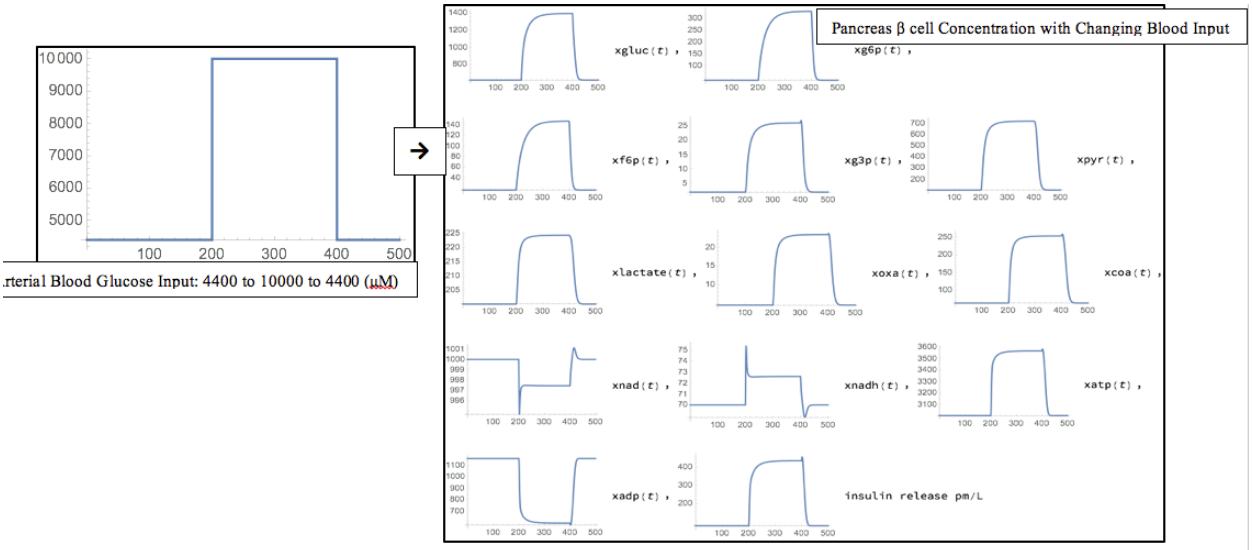


Figure 14.2 3 β cell with glucose input 4.4mM to 10mM to 4.4mM

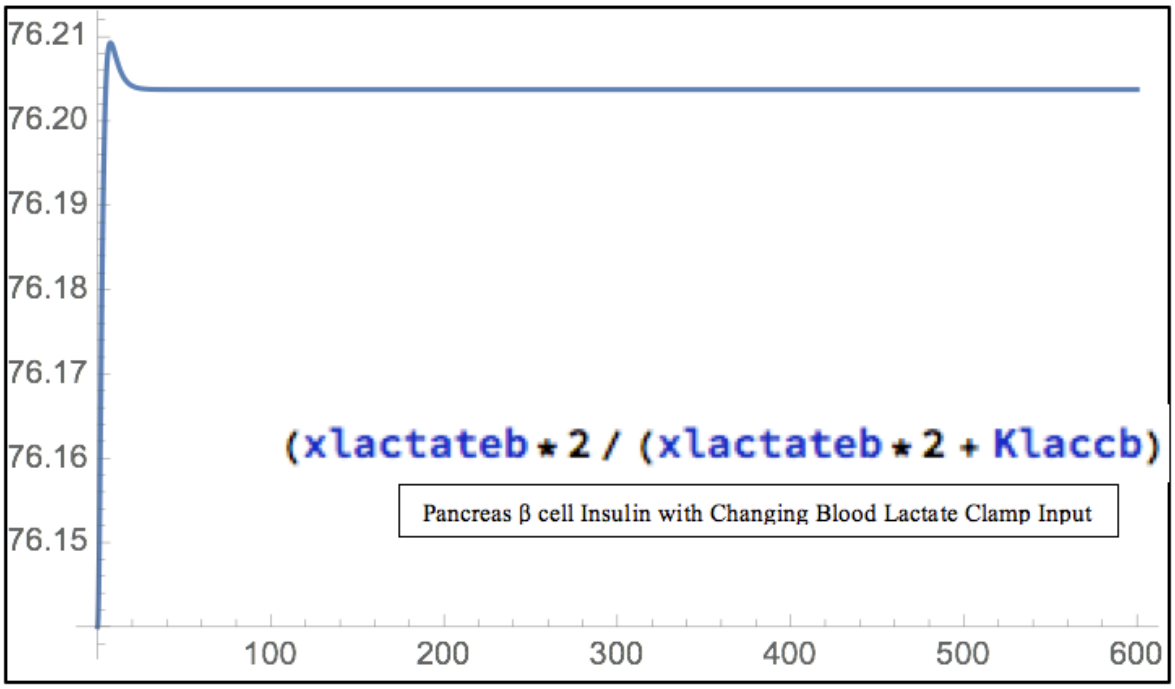


Figure 14.2 4 β cell double lactate clamp

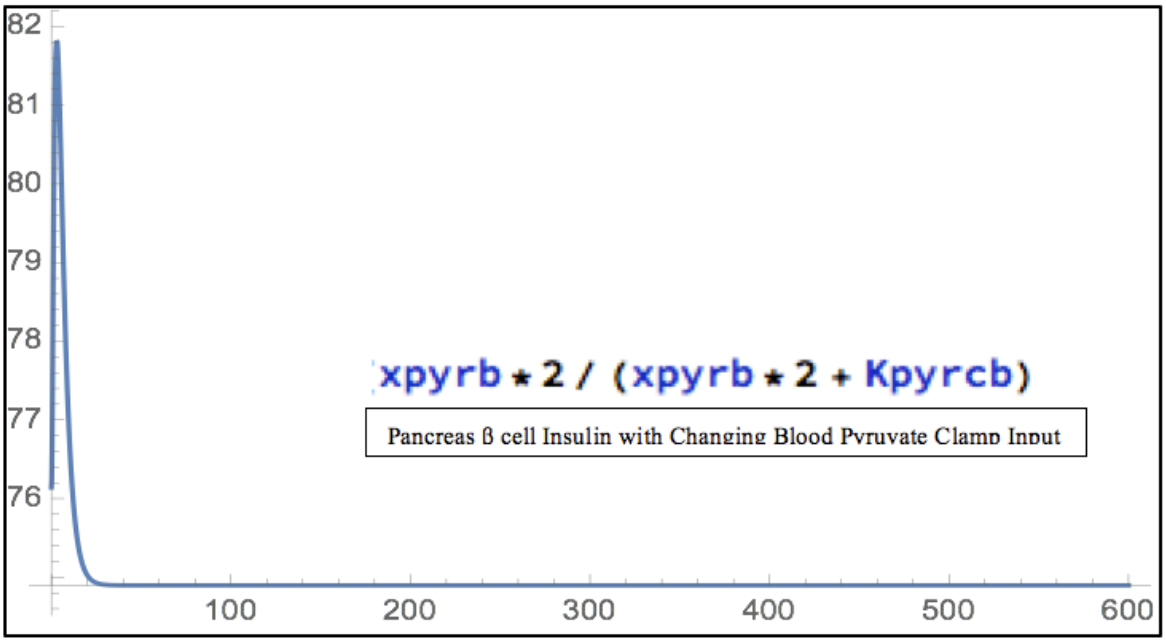


Figure 14.2 5 β cell double pyruvate clamp

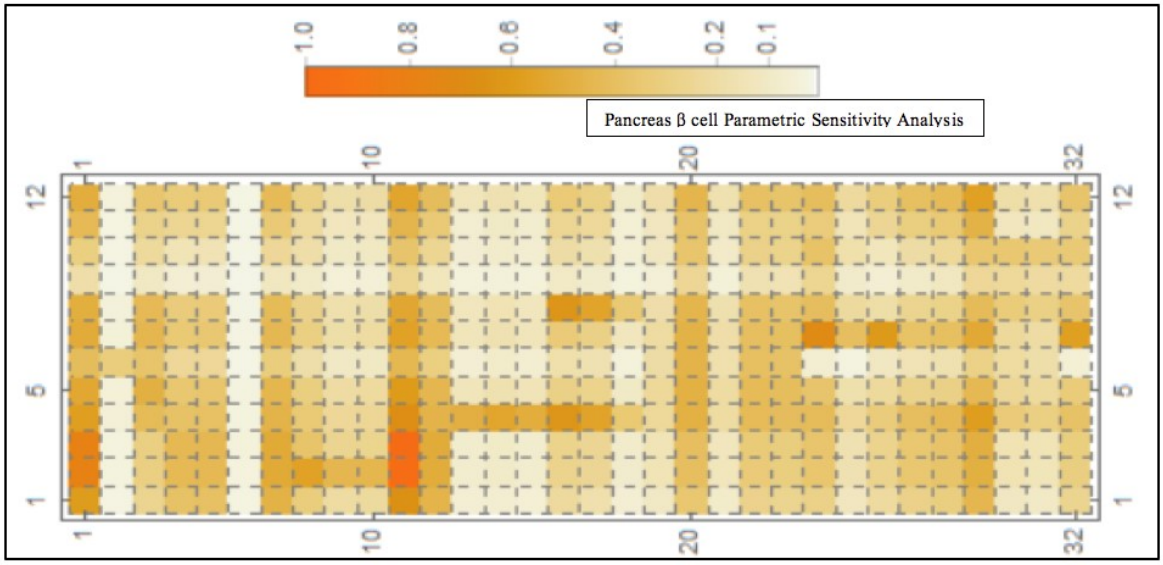


Figure 14.2 6 β cell sensitivity analysis

Figure 14.2.1 is schematics of β Cell metabolism. The pathway is identical to α Cell.

Both figure 14.2.1 and figure 14.2.2 are the steady state. They show what happen to intracellular concentration of metabolites and fluxes during homeostasis. Every concentration and flux stays the same at its steady state value. This is an expected behavior because the blood macronutrient concentration is kept constant by infusion.

Figure 14.2.3 shows the optimized model reacting to glucose concentration. The input of glucose before 200 seconds are 4400mM. From 200 to 400, glucose concentration goes up to 10000 and falls back to normal after 400. The insulin release rate changes as the result of blood glucose concentration change. The insulin release rate fits the experiment data perfectly.

Both Figure 14.2.4 and figure 14.2.5 are the validation results of the model. Showing that insulin release rate is not affect by either blood lactate or pyruvate concentration. This is pretty ideal and

match the mammal's experiment. This is also supposed to be true for human. In real case, exercise releases lots of lactate, which should not affect insulin release rate.

In both cases, the model simulates the concentration of lactate or pyruvate equals twice as steady state. The insulin release rate almost does not change at all for both cases.

14.3 Liver

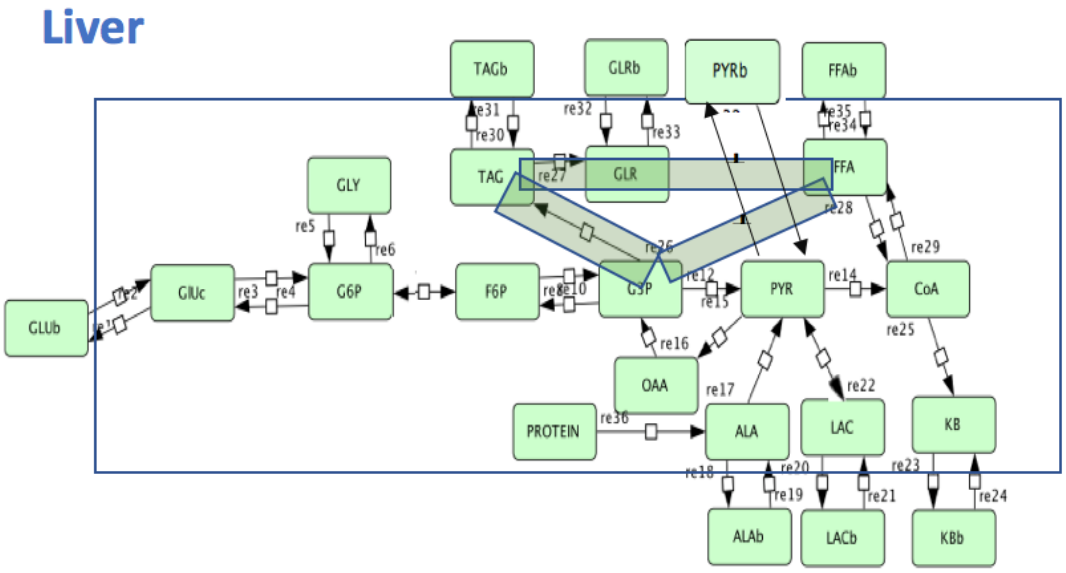


Figure 14.3 1 Liver metabolic pathway

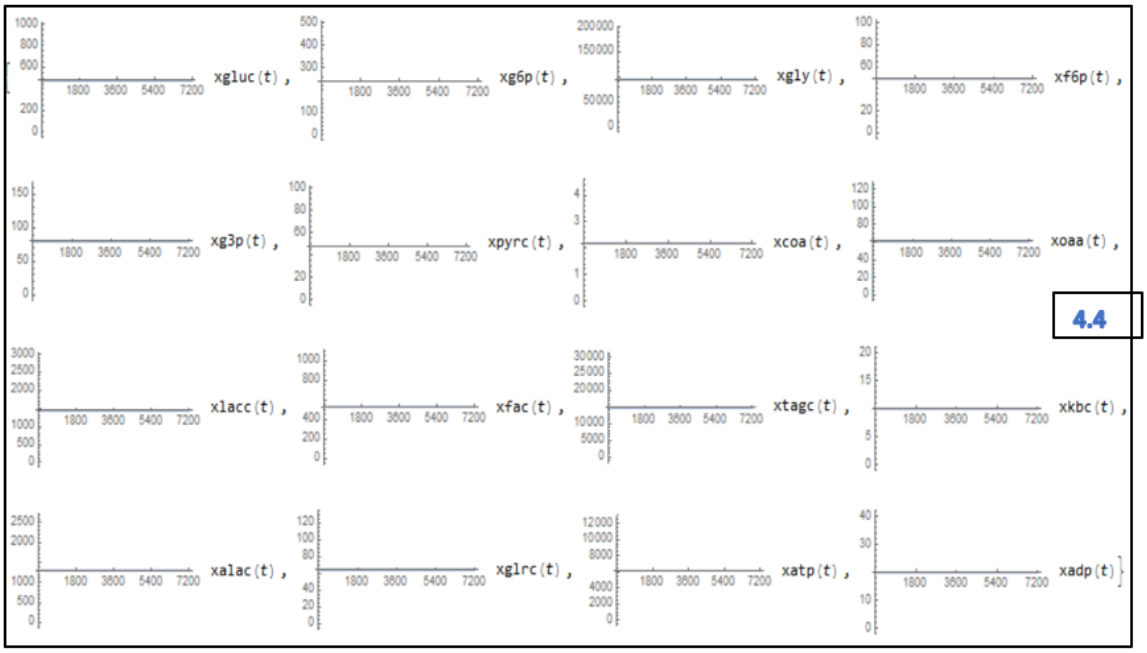


Figure 14.3 2 Liver steady state

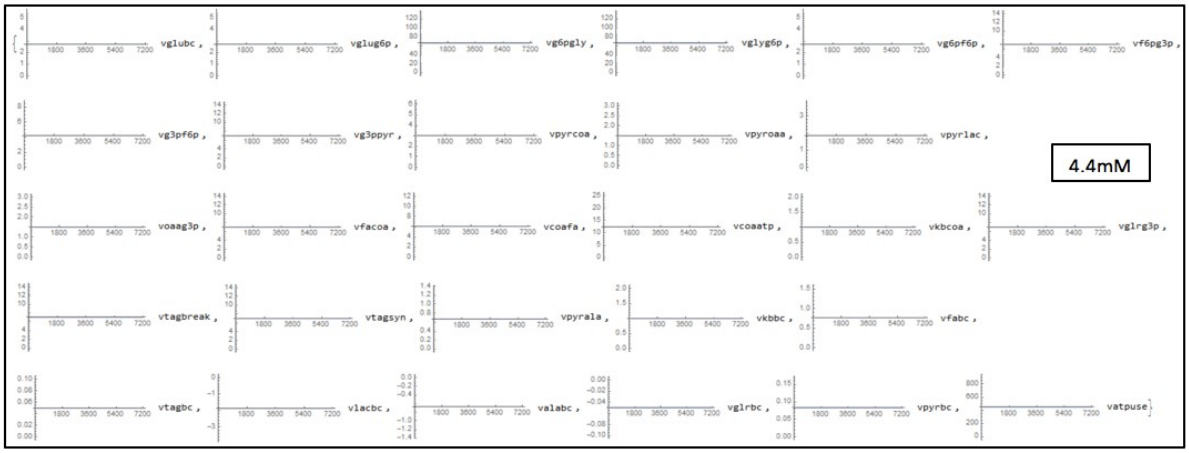


Figure 14.3 3 Liver steady state

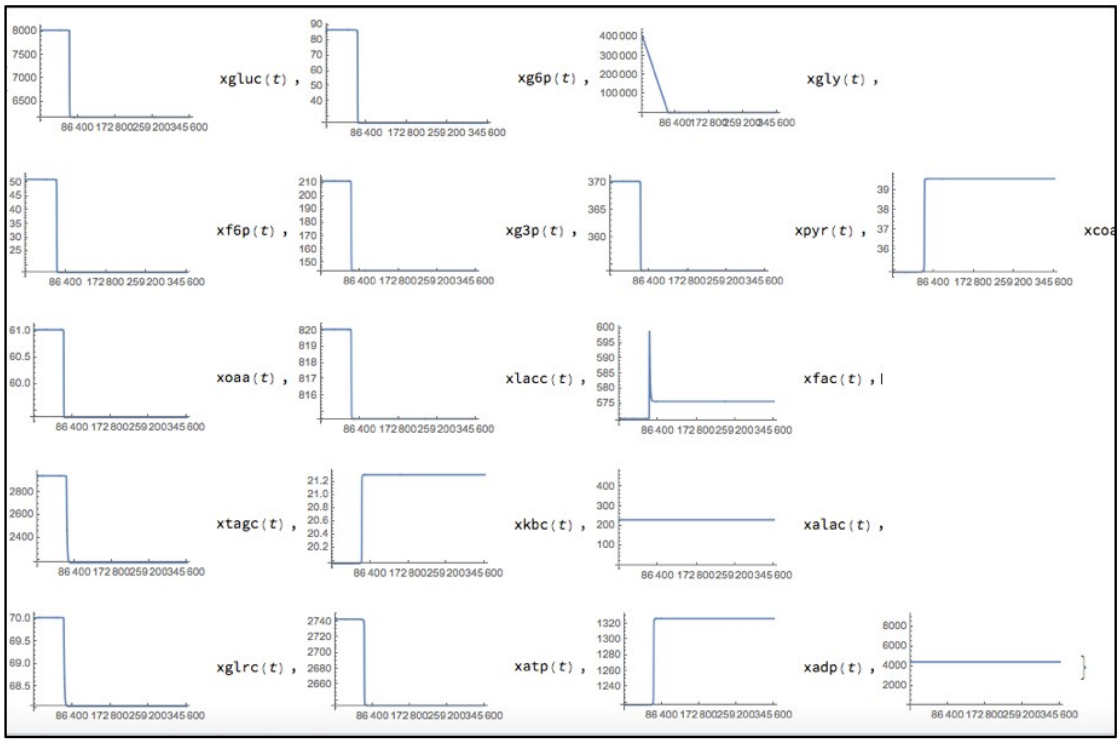


Figure 14.3 4 Liver revised steady state

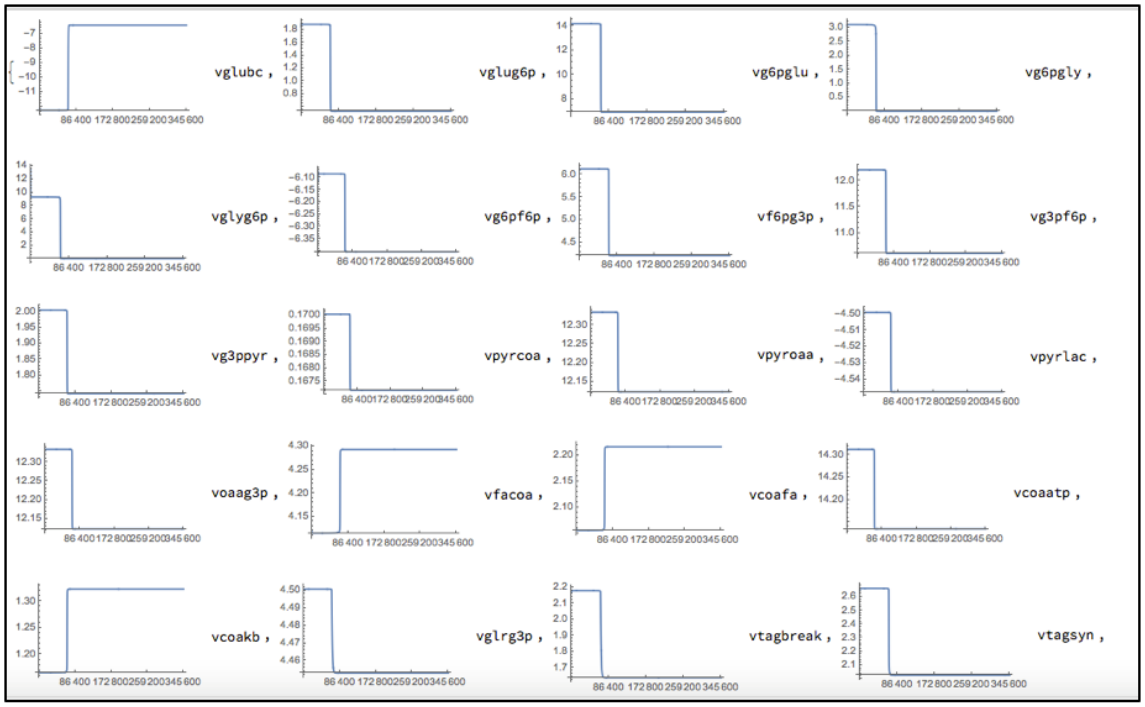


Figure 14.3 5 Liver revised steady state

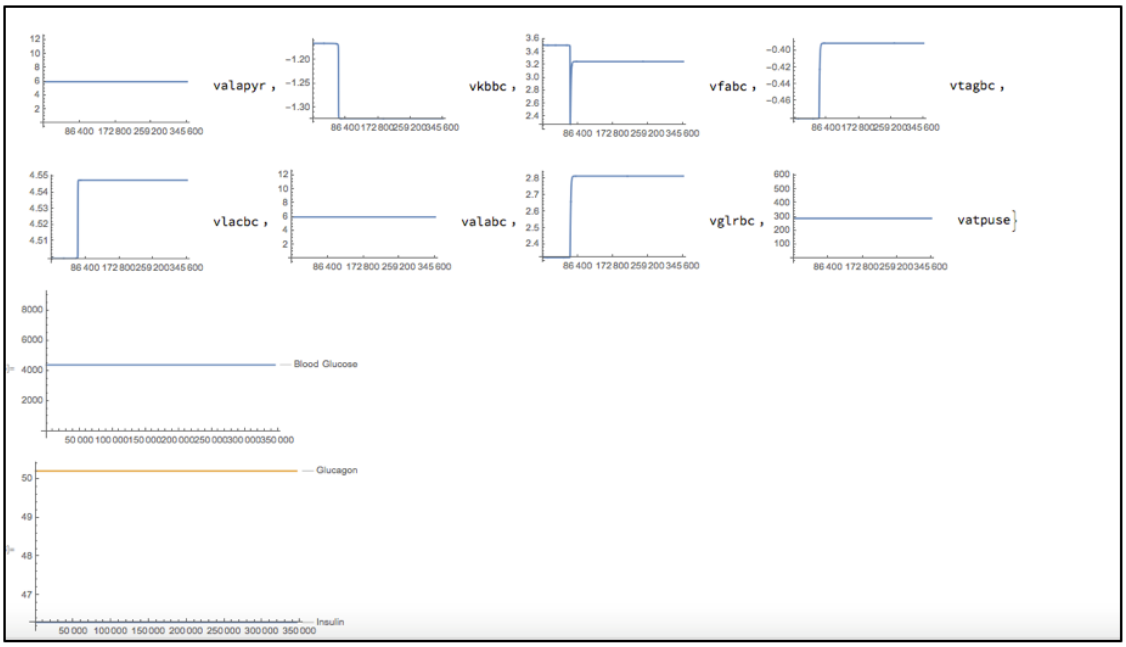


Figure 14.3 6 Liver revised steady state

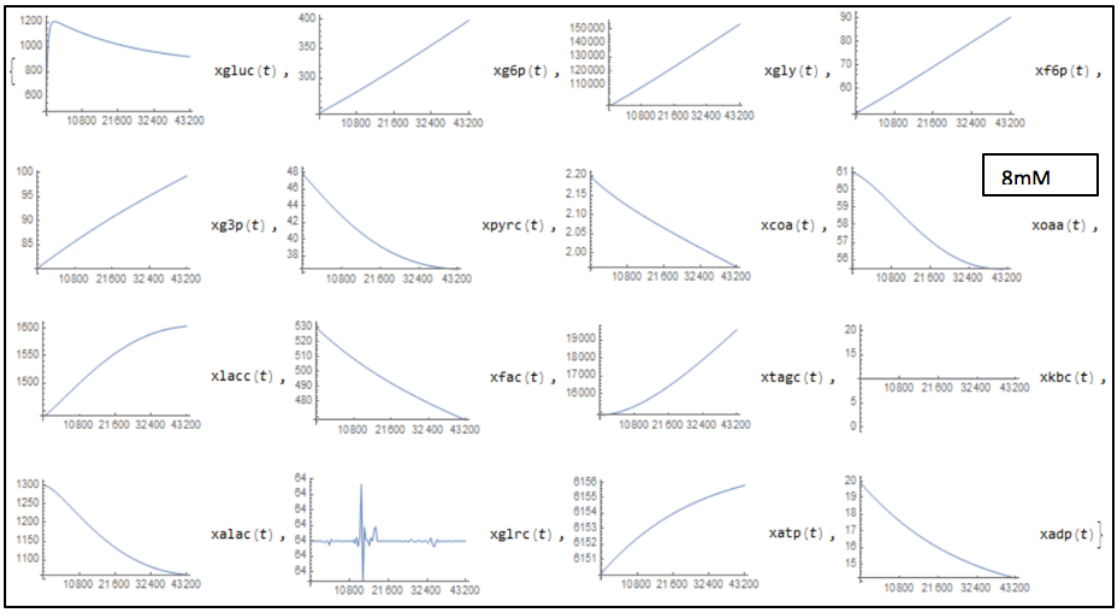


Figure 14.3 7 Liver 8mM glucose clamp

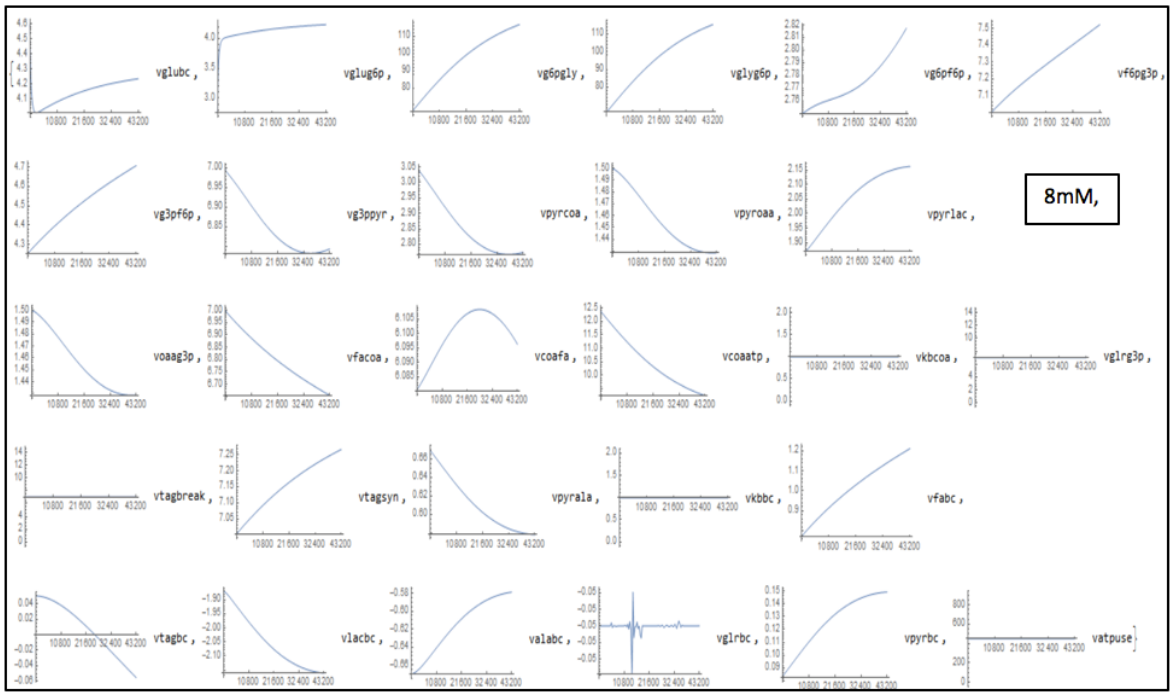


Figure 14.3 8 Liver 8mM glucose clamp

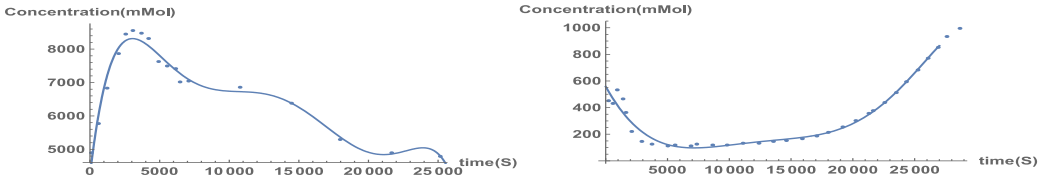


Figure 14.3 9 Postprandial glucose and fatty acid profiles

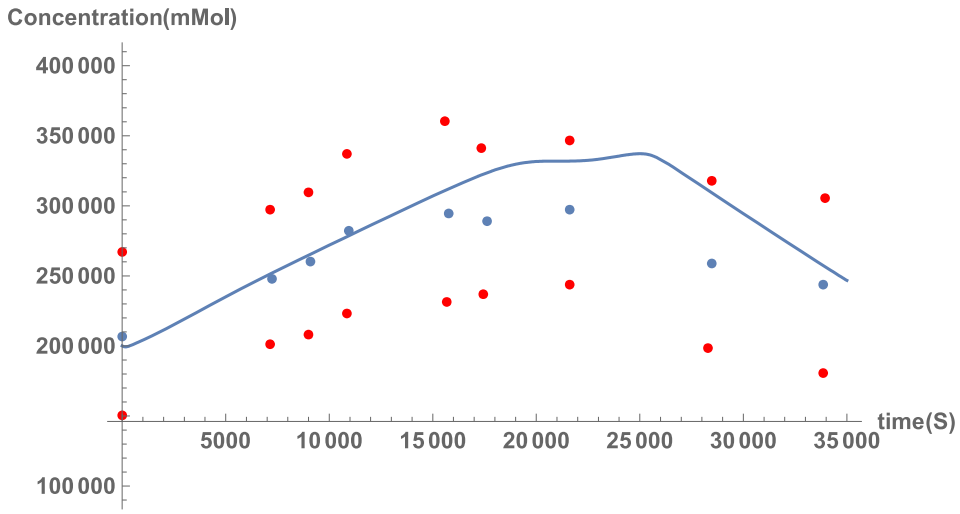


Figure 14.3 10 Optimized glycogen simulation result

Figure 14.3.1 is schematics of liver metabolism. As stated in previous chapters, liver has many fluxes going in and out.

Both figure 14.3.2 and figure 14.3.3 are the steady state set up just like other organs which later on turn out to be not reasonable. They show what happen to intracellular concentration of metabolites and fluxes during homeostasis. Every concentration and flux stays the same at its steady state value. This is an expected behavior because the blood macronutrient concentration is kept constant by infusion.

Figure 14.3.4, figure 14.3.5 and 14.3.6 are revised steady state plots. Liver maintains a steady state by consuming liver glycogen after fasting for 10 hours. Glycogen concentration should keep going down instead of staying the same as figure 14.3.2

Both figure 14.3.7 and 14.3.8 show the simulation with high blood glucose concentration with random parameter. The trend of the plots is reasonable. The high blood concentration causes more

glucose to go into the liver. Glycogen keeps shooting up as a result. We can also see that more fat is being accumulated.

Figure 14.3.10 shows the result of the liver model after optimization. Red dots are the upper and lower bounds based on the human liver storage data after a meal from literature data (Taylor, R, 1996). The blue dots are averages. Figure 14.3.9 is the data from the same literature indicating the blood glucose concentrations (left) and free fatty acid (right) concentrations. Data from figure 14.3.9 is used as model inputs. The optimized model glycogen storage result is the blue line in figure 14.3.10, which fall in the right range between the experiment data upper bound and lower bound.

15. INTERACTIVE APPLICATION

```
shinyUI(fluidPage(
  headerPanel("Type 2 Diabetes Treatment Data Analysis"),
  sidebarPanel(
    radioButtons('sep', 'Delimitor in data file',
                 c(Comma=',',Semicolon=';',Tab='\t'),
                 ','),
    fileInput("patient_data_file","Upload a data file"),
    fileInput("T2D_labels","Upload a labels file"),
    tags$p("Make sure the labels file is tab separated and has two columns: one for animal ID('id') and one for group('gp')"),
    textInput("fig1_label", label = p("Figure 1 Label"), value = "Patient History Groups"),
    checkboxInput("outliers", "Include outliers", TRUE),
    tags$p("Outlier(s) removed:"),
    verbatimTextOutput("outliers"),
    radioButtons('bars',"Error bars show:",c("Standard Error"=1,"Standard Deviation"=2))


  ), #sidebarPanel
  mainPanel(
    uiOutput('select_category1'),
    uiOutput('select_category2'),
    tags$p('Significance Summary:'),
    tableOutput('T2Ddata'),
    tags$p('Graphical summary of data:'),
    plotlyOutput("T2Dbar", height = 350, width = 600)
```

Figure 15 1 Interactive application codes

After previous steps are done, one may want to make an application where one can dynamically interact with the graphs, charts etc. With different data of the user's choice fed as input, the output will be accordingly recalculated and show the updated graphs, charts, numbers etc. This can be realized by using any of the interactive functions of some programming languages of choice. In python there are bokeh or dash libraries, in Wolfram Mathematica, there is animate function and in R, there is shiny library.

The given example is R shiny library's example codes. The shiny app can be executed by writing two separate files: ui.R and server.R files, albeit now both ui and server can be incorporated into a single file. ui.R file allows the users to feed input data. Examples of input data could be numbers or item which the users can enter by sliders, radio buttons, dropdown bar etc. server.R file does all the computation of information which ui.R sent. The codes to generate graphs, bars, charts and so on are in server.R file. The difference between merely executing such codes and server.R is better

interactivity between users and the program, a.k.a. application. Making a shiny app once the full body model is complete is being considered.

16. WHAT TO IMPROVE

To further improve our model, we need to add and/or modify the following.

First, we need to add additional hormonal effects such as adrenaline. Adrenaline will introduce extra glucose release effect during exercise.

Second, blood flow rate change should be considered during exercise and hormonal exposure. This way, more precise physiological change can be represented.

Third, exercise effects should be more clear. Exercise effect does not just release hormones but stimulate different nerves for different organs. Moreover, energy decoupling equation should include exercise terms in terms of work rate or % of VO2max.

Fourth, oxygen levels should be added. According to Ashworth (2016), different oxygen level can bring about differentiated enzyme expressions within the same organ (periportal vs pericentral). The oxygen concentration effect may also be used to better explain TCA and exercise.

Fifth, signaling molecules and transcription factors can be added to our ODEs to further make our model accurate. This way, we can better show molecular etiology and long term effects.

Sixth, all the organs models have to be connected with arterial and venous blood flow. This is to achieve this research's goal of making a comprehensive physiological model.

Lastly, make T2D models based on the whole-body model.

17. BIBLIOGRAPHY

Ajmera, I, et al. “The Impact of Mathematical Modeling on the Understanding of Diabetes and Related Complications.” *CPT: Pharmacometrics & Systems Pharmacology*, vol. 2, no. 7, 2013, doi:10.1038/psp.2013.30.

Ashworth, W.b., et al. “P0947 : A Computational Model of Zonation in Hepatic Metabolism: Understanding Zonated Steatosis in NAFLD and Identification of Potential Drug Targets.” *Journal of Hepatology*, vol. 62, 2015, doi:10.1016/s0168-8278(15)31148-x.

Beller, Mathias, et al. “Lipid Droplets: A Dynamic Organelle Moves into Focus.” *FEBS Letters*, vol. 584, no. 11, 2010, pp. 2176–2182., doi:10.1016/j.febslet.2010.03.022.

Bolinder, J., et al. “Rates of Skeletal Muscle and Adipose Tissue Glycerol Release in Nonobese and Obese Subjects.” *Diabetes*, vol. 49, no. 5, Jan. 2000, pp. 797–802., doi:10.2337/diabetes.49.5.797.

Browning, Jeffrey D., et al. “The Effect of Short-Term Fasting on Liver and Skeletal Muscle Lipid, Glucose, and Energy Metabolism in Healthy Women and Men.” *Journal of Lipid Research*, vol. 53, no. 3, Mar. 2011, pp. 577–586., doi:10.1194/jlr.p020867.

Bulik, Sascha, et al. “The Relative Importance of Kinetic Mechanisms and Variable Enzyme Abundances for the Regulation of Hepatic Glucose Metabolism – Insights from Mathematical Modeling.” *BMC Biology*, vol. 14, no. 1, Feb. 2016, doi:10.1186/s12915-016-0237-6.

Cartee, Gregory D. “Mechanisms for Greater Insulin-Stimulated Glucose Uptake in Normal and Insulin-Resistant Skeletal Muscle after Acute Exercise.” *American Journal of Physiology-Endocrinology and Metabolism*, vol. 309, no. 12, 2015, doi:10.1152/ajpendo.00416.2015.

Chasiotis, D., et al. “Regulation of Glycogenolysis in Human Muscle at Rest and during Exercise.” *Journal of Applied Physiology*, vol. 53, no. 3, 1982, pp. 708–715., doi:10.1152/jappl.1982.53.3.708.

Dash, R.k., et al. “Modeling Cellular Metabolism and Energetics in Skeletal Muscle: Large-Scale Parameter Estimation and Sensitivity Analysis.” *IEEE Transactions on Biomedical Engineering*, vol. 55, no. 4, 2008, pp. 1298–1318., doi:10.1109/tbme.2007.913422.

Defronzo, R. A., and D. Tripathy. “Skeletal Muscle Insulin Resistance Is the Primary Defect in Type 2 Diabetes.” *Diabetes Care*, vol. 32, no. suppl_2, 2009, doi:10.2337/dc09-s302.

Dehghan, Mahshid, et al. “Associations of Fats and Carbohydrates with Cardiovascular Disease and Mortality—PURE and Simple? – Authors Reply.” *The Lancet*, vol. 391, no. 10131, 2018, pp. 1681–1682., doi:10.1016/s0140-6736(18)30774-8.

Feist, Adam Michael. “Model-Driven Metabolic Engineering of Escherichia Coli: a Systems Biology Approach.” 2008.

Feng, Rennan, et al. “Free Fatty Acids Profile among Lean, Overweight and Obese Non-Alcoholic Fatty Liver Disease Patients: a Case – Control Study.” *Lipids in Health and Disease*, vol. 16, no. 1, Apr. 2017, doi:10.1186/s12944-017-0551-1.

Ferretti, Guido. “Erratum to: Maximal Oxygen Consumption in Healthy Humans: Theories and Facts.” *European Journal of Applied Physiology*, vol. 115, no. 3, Nov. 2015, pp. 649–649., doi:10.1007/s00421-015-3102-3.

Frayn, Keith N., et al. “Fatty Acid Metabolism in Adipose Tissue, Muscle and Liver in Health and Disease.” *Essays In Biochemistry*, vol. 42, 2006, pp. 89–103., doi:10.1042/bse0420089.

Girgis, Christian M., et al. “Novel Links between HIFs, Type 2 Diabetes, and Metabolic Syndrome.” *Trends in Endocrinology & Metabolism*, vol. 23, no. 8, 2012, pp. 372–380., doi:10.1016/j.tem.2012.05.003.

Gjedde, A, and C Crone. “Blood-Brain Glucose Transfer: Repression in Chronic Hyperglycemia.” *Science*, vol. 214, no. 4519, 1981, pp. 456–457., doi:10.1126/science.7027439.

Gunton, Jenny E., et al. “Loss of ARNT/HIF1β Mediates Altered Gene Expression and Pancreatic-Islet Dysfunction in Human Type 2 Diabetes.” *Cell*, vol. 122, no. 3, 2005, pp. 337–349., doi:10.1016/j.cell.2005.05.027.

Guo, Y., et al. “Lipid Droplets at a Glance.” *Journal of Cell Science*, vol. 122, no. 6, Apr. 2009, pp. 749–752., doi:10.1242/jcs.037630.

Heilbronn, Leonie K., and Bo Liu. “Do Adipose Tissue Macrophages Promote Insulin Resistance or Adipose Tissue Remodelling in Humans?” *Hormone Molecular Biology and Clinical Investigation*, vol. 20, no. 1, Jan. 2014, doi:10.1515/hmbci-2014-0036.

Hetherington, J., et al. “A Composite Computational Model of Liver Glucose Homeostasis. I. Building the Composite Model.” *Journal of The Royal Society Interface*, vol. 9, no. 69, 2011, pp. 689–700., doi:10.1098/rsif.2011.0141.

Karki, S., et al. “Forkhead Box O-1 Modulation Improves Endothelial Insulin Resistance in Human Obesity.” *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 35, no. 6, 2015, pp. 1498–1506., doi:10.1161/atvbaha.114.305139.

Kim, Jaeyeon, et al. “Multi-Scale Computational Model of Fuel Homeostasis During Exercise: Effect of Hormonal Control.” *Annals of Biomedical Engineering*, vol. 35, no. 1, 2006, pp. 69–90., doi:10.1007/s10439-006-9201-x.

Kim, Jaeyeon, et al. “Multi-Scale Computational Model of Fuel Homeostasis During Exercise: Effect of Hormonal Control.” *Annals of Biomedical Engineering*, vol. 35, no. 1, 2006, pp. 69–90., doi:10.1007/s10439-006-9201-x.

Kim, Jaeyeon, et al. “A Computational Model of Adipose Tissue Metabolism: Evidence for Intracellular Compartmentation and Differential Activation of Lipases.” *Journal of Theoretical Biology*, vol. 251, no. 3, 2008, pp. 523–540., doi:10.1016/j.jtbi.2007.12.005.

Kim, Jaeyeon, et al. “Regulation of Adipose Tissue Metabolism in Humans: Analysis of Responses to the Hyperinsulinemic-Euglycemic Clamp Experiment.” *Cellular and Molecular Bioengineering*, vol. 4, no. 2, Aug. 2011, pp. 281–301., doi:10.1007/s12195-011-0162-2.

Li, Yanjun, et al. “Computational Model of Cellular Metabolic Dynamics in Skeletal Muscle Fibers During Moderate Intensity Exercise.” *Cellular and Molecular Bioengineering*, vol. 5, no. 1, 2011, pp. 92–112., doi:10.1007/s12195-011-0210-y.

Lomako, J. “Glycogenin: the Primer for Mammalian and Yeast Glycogen Synthesis.” *Biochimica Et Biophysica Acta (BBA) - General Subjects*, vol. 1673, no. 1-2, 2004, pp. 45–55., doi:10.1016/j.bbagen.2004.03.017.

Loon, Luc J. C. Van, et al. “The Effects of Increasing Exercise Intensity on Muscle Fuel Utilisation in Humans.” *The Journal of Physiology*, vol. 536, no. 1, 2001, pp. 295–304., doi:10.1111/j.1469-7793.2001.00295.x.

Luo, Liping, and Meilian Liu. “Adipose Tissue in Control of Metabolism.” *Journal of Endocrinology*, vol. 231, no. 3, 2016, doi:10.1530/joe-16-0211.

Marchand, I., et al. “Quantification of Subcellular Glycogen in Resting Human Muscle: Granule Size, Number, and Location.” *Journal of Applied Physiology*, vol. 93, no. 5, 2002, pp. 1598–1607., doi:10.1152/japplphysiol.00585.2001.

Marchand, I., et al. “Quantitative Assessment of Human Muscle Glycogen Granules Size and Number in Subcellular Locations during Recovery from Prolonged Exercise.” *The Journal of Physiology*, vol. 580, no. 2, Oct. 2007, pp. 617–628., doi:10.1113/jphysiol.2006.122457.

Matthews, D. R., et al. “Homeostasis Model Assessment: Insulin Resistance and ?-Cell Function from Fasting Plasma Glucose and Insulin Concentrations in Man.” *Diabetologia*, vol. 28, no. 7, 1985, pp. 412–419., doi:10.1007/bf00280883.

Müller, C., et al. “Endogenous Glucose Production, Gluconeogenesis and Liver Glycogen Concentration in Obese Non-Diabetic Patients.” *Diabetologia*, vol. 40, no. 4, 1997, pp. 463–468., doi:10.1007/s001250050701.

Nogiec, Christopher, et al. “Metabolic Modeling of Muscle Metabolism Identifies Key Reactions Linked to Insulin Resistance Phenotypes.” *Molecular Metabolism*, vol. 4, no. 3, 2015, pp. 151–163., doi:10.1016/j.molmet.2014.12.012.

Noguchi, R., et al. “The Selective Control of Glycolysis, Gluconeogenesis and Glycogenesis by Temporal Insulin Patterns.” *Molecular Systems Biology*, vol. 9, no. 1, 2014, pp. 664–664., doi:10.1038/msb.2013.19.

Nookaew, Intawat, et al. “Adipose Tissue Resting Energy Expenditure and Expression of Genes Involved in Mitochondrial Function Are Higher in Women than in Men.” *The Journal of Clinical Endocrinology & Metabolism*, vol. 98, no. 2, 2013, doi:10.1210/jc.2012-2764.

Oldfors, Anders. “Is Glycogenin Essential for Glycogen Synthesis?” *Cell Metabolism*, vol. 26, no. 1, 2017, pp. 12–14., doi:10.1016/j.cmet.2017.06.017.

Palsson, Bernhard Ø. *Systems Biology: Constraint-Based Reconstruction and Analysis*. Cambridge Univ. Pr., 2015.

Pereira, Rodrigo Martins, et al. “Molecular Mechanisms of Glucose Uptake in Skeletal Muscle at Rest and in Response to Exercise.” *Motriz: Revista De Educação Física*, vol. 23, no. spe, 2017, doi:10.1590/s1980-6574201700si0004.

Pillai, Renjitha, et al. “Deletion of ARNT/HIF1β in Pancreatic Beta Cells Does Not Impair Glucose Homeostasis in Mice, but Is Associated with Defective Glucose Sensing Ex Vivo.” *Diabetologia*, vol. 58, no. 12, 2015, pp. 2832–2842., doi:10.1007/s00125-015-3768-4.

Pratt, Adrian C., et al. “Mathematical Modelling of Hepatic Lipid Metabolism, Mathematical Biosciences 262 (2015) 167–181.” *Mathematical Biosciences*, vol. 265, 2015, pp. 101–103., doi:10.1016/j.mbs.2015.05.003.

Rich, Stephen S. “Still a Geneticists Nightmare.” *Nature*, vol. 536, no. 7614, 2016, pp. 37–38., doi:10.1038/nature18906.

Rolfsson, Ottar, et al. “The Human Metabolic Reconstruction Recon 1 Directs Hypotheses of Novel Human Metabolic Functions.” *BMC Systems Biology*, vol. 5, no. 1, 2011, p. 155., doi:10.1186/1752-0509-5-155.

Rosen, Evan D., and Bruce M. Spiegelman. “What We Talk About When We Talk About Fat.” *Cell*, vol. 156, no. 1-2, 2014, pp. 20–44., doi:10.1016/j.cell.2013.12.012.

Rui, Liangyou. “Energy Metabolism in the Liver.” *Comprehensive Physiology*, Oct. 2014, pp. 177–197., doi:10.1002/cphy.c130024.

Schuster, Ronny, and Hermann-Georg Holzhutter. “Use of Mathematical Models for Predicting the Metabolic Effect of Large-Scale Enzyme Activity Alterations. Application to Enzyme Deficiencies of Red Blood Cells.” *European Journal of Biochemistry*, vol. 229, no. 2, 1995, pp. 403–418., doi:10.1111/j.1432-1033.1995.tb20481.x.

Sjöstrand, Mikaela, et al. “Estimations of Muscle Interstitial Insulin, Glucose, and Lactate in Type 2 Diabetic Subjects.” *American Journal of Physiology-Endocrinology and Metabolism*, vol. 279, no. 5, 2000, doi:10.1152/ajpendo.2000.279.5.e1097.

Somvanshi, Pramod R., et al. “Influence of Plasma Macronutrient Levels on Hepatic Metabolism: Role of Regulatory Networks in Homeostasis and Disease States.” *RSC Advances*, vol. 6, no. 17, 2016, pp. 14344–14371., doi:10.1039/c5ra18128c.

Sonntag, Annika G., et al. “A Modelling-Experimental Approach Reveals Insulin Receptor Substrate (IRS)-Dependent Regulation of Adenosine Monosphosphate-Dependent Kinase (AMPK) by Insulin.” *FEBS Journal*, vol. 279, no. 18, Mar. 2012, pp. 3314–3328., doi:10.1111/j.1742-4658.2012.08582.x.

Sorensen, John Thomas. “A Physiologic Model of Glucose Metabolism in Man and Its Use to Design and Assess Improved Insulin Therapies for Diabetes.” 1985.

Stanford, Kristin I., and Laurie J. Goodyear. “Exercise and Type 2 Diabetes: Molecular Mechanisms Regulating Glucose Uptake in Skeletal Muscle.” *Advances in Physiology Education*, vol. 38, no. 4, 2014, pp. 308–314., doi:10.1152/advan.00080.2014.

Stephenson, M. C., et al. “Variability in Fasting Lipid and Glycogen Contents in Hepatic and Skeletal Muscle Tissue in Subjects with and without Type 2 Diabetes: a1H and13C MRS Study.” *NMR in Biomedicine*, vol. 26, no. 11, Sept. 2013, pp. 1518–1526., doi:10.1002/nbm.2985.

Stöckli, Jacqueline, et al. “GLUT4 Exocytosis.” *Journal of Cell Science*, vol. 124, no. 24, 2011, pp. 4147–4159., doi:10.1242/jcs.097063.

Swarbrick, Michael M. “Quantifying the Contribution of ‘Browned’ White Adipose Tissue Depots to Whole-Body Glucose Homeostasis.” *Obesity Research & Clinical Practice*, vol. 7, 2013, doi:10.1016/j.orcp.2013.12.553.

Taylor, R, et al. “Direct Assessment of Liver Glycogen Storage by 13C Nuclear Magnetic Resonance Spectroscopy and Regulation of Glucose Homeostasis after a Mixed Meal in Normal Subjects.” *Journal of Clinical Investigation*, vol. 97, no. 1, Jan. 1996, pp. 126–132., doi:10.1172/jci118379.

Teicholz, Nina. *The Big Fat Surprise*.

Tiran, Joseph, et al. “A Simulation Model of Extracellular Glucose Distribution in the Human Body.” *Annals of Biomedical Engineering*, vol. 3, no. 1, 1975, pp. 34–46., doi:10.1007/bf02584487.

Wang, Xiaohui L., et al. “Ablation of ARNT/HIF1β in Liver Alters Gluconeogenesis, Lipogenic Gene Expression, and Serum Ketones.” *Cell Metabolism*, vol. 9, no. 5, 2009, pp. 428–439., doi:10.1016/j.cmet.2009.04.001.

Weber, J.-M. “Metabolic Fuels: Regulating Fluxes to Select Mix.” *Journal of Experimental Biology*, vol. 214, no. 2, 2010, pp. 286–294., doi:10.1242/jeb.047050.

Xu, Hongyang, et al. “Rat Skeletal Muscle Glycogen Degradation Pathways Reveal Differential Association of Glycogen-Related Proteins with Glycogen Granules.” *Journal of Physiology and Biochemistry*, vol. 71, no. 2, 2015, pp. 267–280., doi:10.1007/s13105-015-0407-y.

Yamaguchi, Miwa, et al. “Association between Brain-Muscle-ARNT-like Protein-2 (BMAL2) Gene Polymorphism and Type 2 Diabetes Mellitus in Obese Japanese Individuals: A Cross-Sectional Analysis of the Japan Multi-Institutional Collaborative Cohort Study.” *Diabetes Research and Clinical Practice*, vol. 110, no. 3, 2015, pp. 301–308., doi:10.1016/j.diabres.2015.10.009.

YIFEI LI

CONTACT INFORMATION

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Date of Birth/Birthplace: November 24th 1993, Beijing, People's Republic of China

EDUCATION BACKGROUND

Sep 2012 - June 2016
Bachelor of Science in Chemistry
Xiamen University, Fujian, China
GPA: 3.4/4 (84.5/100)

Sep 2016 - present
Master of Science in Engineering in Chemical and Biomolecular Engineering
Johns Hopkins University, Baltimore, Maryland
GPA: 3.7/4

RESEARCH EXPERIENCE

Jan 2013 - Jan 2014
Xiamen University Advisor: *Prof. Shunhua Li*
Research project of combination of novel supramolecular fluorescence sensor via hydrogen-bonding self-assembly

Skills: Performed organic chemistry experiment; Used techniques such as TLC, column chromatography, and NMR, LC-MS to separate, refine and identify products

Jul 2014 - Sep 2014
Chinese Academy of Agricultural Sciences Advisor: *Prof. Kongming Wu*
Research project of molecular cloning and characterization of novel stress-responsive gene and ferric reductases gene

Skills: Performed purification of DNA, construction of eukaryotic expression vector. Used techniques such as PCR, software in bioinformatics like vector NTI suit.

Sep 2015 - Jul 2016
Xiamen University Advisor: *Prof. Yufen Zhao*
Research project on N-phosphono-amino acids

Skills: Mastered the skills of isolating and purifying a protein of interest; Optimized reaction conditions and chose synthetic routes by myself; Used techniques such as western bolt, ELISA.

Nov 2016 - present
Johns Hopkins University Advisor: *Prof. Marc D. Donohue*
Research project on Pharmacokinetic and Pharmacodynamic (PKPD) modeling on macronutrients (glucose, fat, protein etc.) and metabolites (G6P, F6P, glycogen etc.) in healthy human and type 2 diabetic patients.

Skills: Built mathematical whole body and organ specific models based on collected human physiology and molecular mechanism data; Set ordinary differential equations (ODEs) using metabolic flux expressions of Michaelis-Menten and kinetic equations; Performed flux balance analysis (FBA) for homeostatic analysis; Computed underdetermined system fluxes using objective functions with multiple constraint conditions; Programmed in Mathematica, MATLAB and R for simulation and analysis to figure out concentration, flux and other relevant information; Used different optimizing techniques to optimize model such as non-linear square minimization for parameter finding

Academic Publications

• Pimpan Pimonrat, Xiaomin Liu, Pengwei Sun, Huiming Guo, **Yifei Li,** Hongmei Cheng, *Molecular cloning and characterization of a novel stress-responsive gene, AmHP40, from Ammopipthanthus mongolicus*, Plant Cell, Tissue and Organ Culture (PCTOC): Journal of Plant Biotechnology, Submitted.

• **Yifei Li**,*The Pesticide and Fertilizer Industry and its Related Legal Regulations in the US*, Pesticide Science and Administration, Jan 5th 2015, Page 20 Article No.1002-5480(2016)01-0020-03, Language of publication: Chinese

• Yuqiong Hao, Guoqing Lu, Chunling Wang, **Yifei Li,** Huiming Guo and Hongmei Cheng, *Overexpression of AmDUF1517 enhanced tolerance to various stresses in transgenic cotton* , Journal of Integrative Agriculture, invited paper, Submitted

Computer Skills

Classes taken

• *Language C* (Learned basic grammar)

• *Computational Medicine* (Learned and did the projects in signal processing, image processing, machine learning, and statistical applications to Medical field.)

• *Introduction to Scientific Computing in BME using Python, Matlab, and R*

• *Computational Protein Structure Prediction and Design* (Learned Pyrosetta.)

Research project

• Application of Matlab, R, Mathematica in System biology. Simulated, built math model, optimized parameters in a complex kinetic system.

• Data mining and data visualization.

Others

• Python (Basic grammar, Web crawling technic. Used python to solve simple algorithm problem.)

• Latex (Used latex to edit report and CV.)

Computational projects

• **Cardiac output model**: Used arterial blood pressure data from MIMIC II Clinical Database (Multi parameter Intelligent Monitoring in Intensive Care) to build a cardiac output model predict cardiac output. Reproduced figures from Sun et al (2009), Crit Care Med 37(1): 72.

• **Sepsis model**: Used clinical data in MIMIC II Clinical Database (Multiparameter Intelligent Monitoring in Intensive Care) to build a predictive sepsis model. Classified sepsis based on predictive models. Katharine E. Henry,(2015). Science Translational Medicine, Vol 7, Issue 299.

• **Image processing**: Aligned an atlas amygdala and hippocampus to a target amygdala and hippocampus. Estimated 9 degrees of freedom linear transformation matrix. Then a spline based transformation was estimated and applied to the linearly transformed data.

• **Statistical connectomics**: Tested whether the two populations were different by performing a permutation test and reporting the p-value.

HONORS

Apr 2015	Won the Feiyang Scholarship from Pujing Chemical Co.
Feb 2015	Won the Scholarship for Physical Chemistry Course (1/167)
Jun 2013	Won the fifth place in the women 500 meters dragon boat competition in the Cross Taiwan Strait Dragon Boat Competition
Oct 2013	Won the 9th place in the mixed 500 meters dragon boat competition in the Chinese Talent Dragon Boat Competition
Dec 2013	Won the 5th place in the "Airport Cup" Tennis Group Competition
Oct 2012	Won the championship in the tennis competition for entrants

CAMPUS ACTIVITY

2014-2015	President of the Tennis Club in Xiamen University
Aug 2014	Team leader of the social investigation team in Xiamen Yuxin Diamond Tool Co. Ltd.
2013-2014	Leader of the Dragon Boat Team in Xiamen University
2012-2013	Secretary of the Sports Division of the Student Union
Aug 2012	Attended the EPA 2b Course of "English for Academic Purpose in Eynesbury Language Center, Australia

INTERNSHIP EXPERIENCE

Feb 2015	Interned in Guangxi Tianyuan Biochemical Co. Ltd. searching and classifying materials in English for the company

ENGLISH PROFICIENCY

Oct 2015	TOEFL: 102 (R:30; L:26; S: 22; W:24)
Aug 2015	GRE: 325 (Reading: 155 Math: 170 Writing:3)

INTERESTS AND HOBBIES

Playing tennis, reading, etc.